Neurocognitive impairment following central nervous system infections in Kenyan children as detected by event related potentials

Thesis

UNRESTRICTED

NEUROCOGNITIVE IMPAIRMENT FOLLOWING CENTRAL NERVOUS SYSTEM INFECTIONS IN KENYAN CHILDREN AS DETECTED BY EVENT RELATED POTENTIALS

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Abstract

As mortality in childhood decreases due to advances in modern medicine, presence of better nutrition and fresh water supply, the impact of disability has become increasingly important especially in resource poor countries. Children living in sub-Saharan Africa are also exposed to a number of potentially debilitating infections which have been shown to have long-term cognitive effects even in absence of clinical neurological sequelae. The objective of the study is to demonstrate that event related potentials (ERPs) can be used to detect neurocognitive impairment following the most common central nervous (CNS) system infections affecting children in sub-Saharan Africa, namely falciparum malaria, acute bacterial meningitis (ABM) and human immune-deficiency virus (HIV). Four groups of children were recruited: children previously admitted with severe falciparum malaria (n= 50), or acute bacterial meningitis (n = 65), or HIV-infected (n= 39) or were unexposed to any of these conditions (n= 177). Passive auditory and visual oddball ERP protocols were used.

The results of the group of 50 children aged 6-7 years old with a history of severe falciparum malaria (cerebral malaria, CM= 27, malaria plus seizures, M/S= 14 and prostrated malaria, PM= 9) show that children exposed to CM, M/S and PM had significantly longer auditory N200 and P3a latencies and smaller N200 amplitudes than study controls.

The results of 65 children aged between 4-15 years old with a history of pneumococcal meningitis showed that children with a history of bacterial

meningitis had significantly smaller auditory P100 amplitudes, longer N200 latencies and longer visual P200 latencies than community controls.

Finally, the results of 40 children aged between 18-40 months infected with HIV showed that they had longer auditory P100 latencies, larger auditory P200 amplitudes and smaller Negative component, Nc, amplitudes than community controls.

It is concluded that the CNS infections may result in neuro-developmental delays in childhood. Further, CNS infections may interfere with normal education outcomes by precipitating attention deficit amongst children post infection.

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Contributions:

1. Michael Kihara: I prepared the proposal, was in-charge of study design and methodology, development of ERP paradigms, recorded ERPs and analysed them, trained team members on consent process, ERP acquisition. I analysed data, interpreted it and wrote up the dissertation.
2. Charles RJC Newton: He was the study supervisor and director of studies. He helped in the study design, methodology, data analysis, and interpretation, generating of cases and controls, and revision of the thesis.
3. Michelle de Haan: She was my supervisor and ERP expert. She helped in the development of the experimental paradigms, analysis of ERPs, interpretation of the data and revision of the thesis.
4. Brian GR Neville: He was my supervisor and helped in the revision of the thesis.
5. Harrun Garrashi: He assisted me in the recording of ERPs.
6. Judy Tumaini: She also assisted with recording of ERPs, screening for vision and audition, and maintenance of participant files.
7. Edwin Chengo: He assisted with taking the clinical histories of the patient groups and ascertained whether they met inclusion criteria.
8. Rachel Odhiambo: She transferred all the data from the patient files into spreadsheets.
9. Francis Yaah: He co-ordinated the field team that recruited and consented participants in their homesteads.

ERP Neuro team

From left: Eddie Chengo, Francis Yaah, Racheal Odhiambo, Judy Tumaini, Harrun Garrashi and Michael Kihara.

DEDICATION

To Mercy and "the Boys"

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CHAPTER 1

Brain Insults in Children

1.1. Introduction

1.1.1. Brain insults in children living in Developing Countries

As mortality in childhood decreases due to advances in modern medicine, presence of better nutrition and fresh water supply, the impact of disability has become increasingly important especially in resource poor countries (WHO, 2005). Over 80% of the world's population live in developing countries where it is estimated that half the population is less than 15 years old. In these regions, it is estimated that there are over 200 million (about 4% of the population) children under the age of 5 years old who have cognitive limitations (Grantham-McGregor et al., 2007; Olness, 2003). The causes of this include malnutrition, genetic diseases, infectious diseases, *in-utero* drug and alcohol exposure, birth defects and human immune-deficiency virus (HIV). Children living in sub-Saharan Africa are also exposed to numerous infections, many of which have been shown to have long-term cognitive effects.

Infectious diseases cause more than 13 million deaths per year, mostly of infants and young children with 50% occurring in developing countries (WHO, 2002a). A World Health Organization (WHO) report on infectious diseases estimates that pneumonia, tuberculosis, diarrhoeal diseases, malaria, measles and HIV account for half of all premature deaths (WHO, 1999). Infections of the central nervous system (CNS) are an important cause of neurological deficits and cognitive impairment after the neonatal period (Carter, Mung'ala-Odera et al., 2005; Holding, Stevenson,

Peshu, & Marsh, 1999; Newton, 2001). In developing countries, especially in Africa, the morbid consequences of infectious diseases are often overlooked due to high mortality rates (Carter, 2002). Among children who recover without clinically apparent learning disability or motor impairment, cognitive impairment may be undetected. The morbid consequences may have a devastating impact on the development of an individual child and the general population of a country.

Because of the apparent high frequency of cognitive impairments following infectious diseases, the aims of this study are to investigate cognitive impairment in children who have recovered after exposure to severe falciparum malaria, acute bacterial meningitis (ABM) or who are HIV positive since they are the most common CNS infections of children in this region. There have been recent reviews of the neurocognitive impairments post ABM (Peltola, 2001) and HIV infections (Abubakar, Van Baar, Van de Vijver, Holding, & Newton, 2008) which I summarize, but since there was no review of the effect of *P. falciparum* on cognition, I present the findings in more detail.

1.2. Acute Bacterial Meningitis (ABM)

1.2.1. *Introduction*

Acute bacterial meningitis is a potentially life-threatening infectious disease involving inflammation of the meningeal membranes surrounding the brain that is caused by bacteria. It is most common in infancy and is recognized as a major cause of death among children aged less than 5 years old. In sub-Saharan Africa over 200,000 children aged below 5 years old die of ABM annually and a large proportion of those who survive develop neurological sequelae (Peltola, 2001).

The most common etiologic agents of bacterial meningitis in childhood are *Haemophilus influenza* Type b (Hib), *Neisseria meningitidis* and *Streptococcus pneumoniae* (Hodgson et al., 2001; Wright & Ford, 1995). The Hib conjugate vaccine of haemophilus meningitis has reduced the incidence considerably. Two species of bacteria, *Neisseria meningitidis* and *Streptococcus pneumoniae,* account for up to 85% of ABM (Bohr et al., 1983). Over 50% of cases of meningococcal meningitis occur in Sahelian Africa (the meningococcal belt) where it occurs in epidemics every 8 – 12 years (Hodgson et al., 2001). It is less common outside those areas, and is rarely seen along the Kenyan Coast (Scott et al., 2005).

Streptococcus pneumoniae is the most commonly isolated organism in cerebrospinal fluid (WHO, 1999) and is also the main cause of bacterial meningitis in African countries outside the meningococcal belt (Peltola, 2001) since the introduction of the Hib vaccine. *S. pneumoniae* is a common cause of meningitis in children worldwide and is associated with higher morbidity and mortality than other microbes (Bedford et al., 2001). It is the most commonly isolated organism and responsible for 24% of all meningitis in children admitted at a Kilifi District Hospital in Kenya between 1996 and 2002 (Mwangi et al., 2002).

1.2.2 *Epidemiology*

In 2005, WHO estimated that 1.6 million deaths were caused by *S. pneumoniae* annually; this estimate includes the deaths of 0.7–1 million children aged <5 years old. Most of these deaths occur in developing countries (WHO, 2007), and children aged <2 years old are disproportionately represented among these deaths. In Europe

and the United States, *S. pneumoniae* is the most common cause of community-acquired bacterial pneumonia in adults. In these regions, the annual incidence of invasive pneumococcal disease ranges from 10 to 100 cases per 100 000 population (WHO, 2007).

In Kenya since the introduction of the Hib vaccine, the proportion of *S. pneumoniae* has risen to 52% of all meningitis. In Kenyatta National Hospital, *S. pneumoniae* was associated with a mortality of 40%, while 60% of the survivors had neurological deficits (Were, 2007). Most hospital based data do not provide a reliable estimate of the burden of morbidity and mortality resulting from meningitis, since some neurological deficits recover with time whilst other neurocognitive deficits are difficult to detect on discharge e.g. hearing loss and learning deficits (Carter, Mung'ala-Odera et al., 2005).

1.2.3. *Neurocognitive sequelae post ABM*

Prior to the advent of antibiotics in the 1950's, over 90% of the children who contracted bacterial meningitis did not survive the acute illness (Adams et al., 1993). In spite of vaccines, antibiotics and advanced management of critically ill, there remains a significant risk of death and severe neurological sequelae following bacterial meningitis in childhood. Further, the increased survival of children has raised concerns about the morbid consequences of ABM. The extent of the long-term morbidity is less well known, particularly in sub-Saharan Africa.

The consequences of bacterial meningitis in infancy may vary from no sequelae through to death. Neurocognitive sequelae have been identified in many survivors, but the type and frequency of neurocognitive outcomes have varied across studies.

Neurological impairments associated with ABM include motor deficits, hearing loss, visual disorders, epilepsy and behavioural problems (Anderson, Anderson, Grimwood, & Nolan, 2004; Grimwood et al., 1995). The intellectual functions of children post bacterial meningitis may be impaired (Anderson et al., 2004; Anderson et al., 1997; Grimwood, Anderson, Anderson, Tan, & Nolan, 2000; H. G. Taylor, Barry, & Schatschneider, 1993; H. G. Taylor et al., 1997). Cognitive deficiencies include learning difficulties, short-term memory deficits and poor academic performance (Bedford et al., 2001; Merkelbach, Sittinger, Schweizer, & Muller, 2000; Schmidt et al., 2006). Impairments in cognitive functions have been reported in areas of auditory perception and language functions (Anderson et al., 1997; Bedford et al., 2001), intelligence (H. G. Taylor, Schatschneider, & Minich, 2000), reading (Grimwood et al., 1995), executive functions (Grimwood et al., 1995; H. G. Taylor et al., 2000; H. G. Taylor, Schatschneider, Petrill, Barry, & Owens, 1996) and have been shown to exist up to 12 years post infection (Anderson et al., 2004). However, it seems that children whose disease course was free of complications had lower risk of poor outcome than those with complications (Koomen et al., 2004; H. G. Taylor et al., 2000).

Cognitive impairment has been detected in 12 – 29% of survivors in studies of ABM in the West (Bohr et al., 1985; Fellick et al., 2001; Franco, Cornelius, & Andrews, 1992; Merkelbach et al., 2000; Stevens, Earnes, Kent, Holt, & Harvey, 2003; H. G. Taylor et al., 1997) but there are few data from Africa. Psychometric findings of cognitive functions showed decreased visual memory, reduced concentration, a reduction in visuo-constructive capacity and diminished logical memory (Merkelbach et al., 2000). A prospective 7-year follow up study of

children with a history of bacterial meningitis showed lower intelligence scores (IQ) and poorer performance in neuropsychological tasks than there peers (Grimwood et al., 1995). A follow-up of this cohort 12 years after the meningitis episode demonstrated that cognitive deficits in problem solving, verbal fluency, organizational skills and mental flexibility persist over time (Grimwood et al., 2000).

1.3. Human Immunodeficiency Virus (HIV)

1.3.1. *Introduction*

Human Immunodeficiency Virus (HIV) is the retrovirus that causes acquired immuno-deficiency syndrome (AIDS), a condition in humans in which the immune system is impaired, leading to life-threatening opportunistic infections. It invades the CNS directly and increases the susceptibility to CNS infections. Vertical transmission is the main mode of infection among children. This mother-to-child transmission can occur *in utero*, at birth or after birth through breast feeding (UNAIDS, 2004). In African children, breastfeeding accounts for up to 20% vertically infected children (Dabis & Ekpini, 2002). Sub-Saharan Africa bears the brunt of the global epidemic, with HIV retarding economic growth and increasing poverty. In the past, the prognosis of HIV-infected children was poor with over 50% dying before their second birthday (Newell et al., 2004). The recent advances in provision of highly active antiretroviral treatment (HAART) are likely to decrease mortality rates but may increase HIV-related morbidity.

1.3.2. *Epidemiology*

It is estimated that about 0.6% of the world's population is infected with HIV (Joint-United-Nations-Programme-on-HIV/AIDS, 2006). A total of 39.5 million [34.1million–47.1 million] people were living with HIV in 2006 (UNAIDS, 2006). In 2006, AIDS claimed an estimated 1.8–2.4 million lives, of which more than 570,000 were children. Two-thirds (63%) of all HIV cases globally live in sub-Saharan Africa (SSA) mostly concentrated in southern Africa. Africa is home to 95% of all mother-to-child transmissions of HIV (Quinn, 1998; UNAIDS, 2004). According to current estimates, HIV is set to infect 90 million people in Africa, resulting in a minimum estimate of 18 million orphans by the year 2010 (Joint-United-Nations-Programme-on-HIV/AIDS, 2006). The spread of HIV has reversed progress in health, education, life expectancy, and standards of living that Africa has made since the 1950s (Meel, 2003).

1.3.3. *Neurocognitive effects of HIV infection*

The HIV-AIDS pandemic is potentially a major cause of developmental disabilities in Africa. Neurocognitive deficits associated with HIV may occur at any age depending on the time of exposure to the virus. The cognitive consequences of HIV are often confounded with other stressors and conditions that impede neurodevelopment. These include factors such as poverty, lack of stimulation, drug exposure, malnutrition or possible death/illness of parent (Willen, 2006). Early exposure to HIV infection has been related to impairment in infant neuro-developmental functioning (in the domains of cognitive, motor and language functioning) (Chase et al., 2000; Coplan et al., 1998; Nozyce et al., 2006; R. Smith et al., 2000; Wolters, Brouwers, Civitello, & Moss, 1997). A critical pathway to

impairment appears to be CNS involvement (Blanchette, Smith, King, Fernandes-Penney, & Read, 2002; Cabre, Smadja, Cabie, & Newton, 2000; W. G. Knight, Mellins, Levenson, Arpadi, & Kairam, 2000), but the exact relationship between exposure and outcome appears to depend upon age, biological and environmental risk factors (Coscia, Christensen, & Henry, 1997; Lindsey, O'Donnell, & Brouwers, 2000).

The effects of vertically transmitted HIV on a child's neuro-development can range from mild to devastating. Studies have found that neuro-developmental delays may begin as early as 4 months of age and continue into school years. Children with HIV infection are often exposed to environmental factors that impair normal development including stigmatization, unstable or multiple caregivers, and poverty (WHO, 2006).

The developmental deficits as a result of HIV infection in children include language, memory, visual spatial skills and executive function (Bisiacchi, Suppiej, & Laverda, 2000). Executive function is difficult to assess in young children, but the "A not B" test has detected impairments in infants (Noland, Singer, Mehta, & Super, 2003). Some studies have shown an increased prevalence of cognitive impairment among HIV infected children compared to those uninfected (Armstrong, Seidel, & Swales, 1993; Blanchette et al., 2002; Chase et al., 2000). Other studies have shown that there are no differences between infected and non-infected preschool-age children (Fishkin et al., 2000). A study in Uganda of HIV-infected children, aged 6-12 years old, did not find significant cognitive differences with unexposed children (Bagenda et al., 2006). However, another study found that

children with HIV infection and had suffered from an AIDS-defining illness performed significantly poorer in a cognitive test than those infected but without illness or non-infected children (R. Smith et al., 2006).

The use of combined anti-retroviral therapies have markedly improved immune function and improved cognitive development in some studies (Ferrando, Rabkin, van Gorp, Lin, & McElhiney, 2003; Robertson et al., 2004) but not in others (Tamula, Wolters, Walsek, Zeichner, & Civitello, 2003). However, these therapies are yet to be used widely in resource poor settings, such as SSA and hence children exposed to HIV infection in the region may have poorer cognitive performance than their peers.

1.4. Falciparum Malaria

1.4.1. *Introduction*

Malaria is caused by an infection with protozoa of the genus *Plasmodium.* The most serious forms of the disease are caused by *Plasmodium falciparum* and *Plasmodium vivax,* but other related species *(Plasmodium ovale, Plasmodium malariae, and* sometimes *Plasmodium knowlesi)* can also infect humans. The name "malaria" was derived from Italian *"male aria"* which means "bad air", as malaria was thought to be caused by the poisonous gases from swamps (Capanna, 2006). Malaria parasites are transmitted by female *Anopheles* mosquitoes which require blood meals to produce eggs. The parasites multiply within red blood cells of humans, causing symptoms that include symptoms of fever, chills, anaemia (light headedness, shortness of breath, tachycardia etc.), as well as other general symptoms such as nausea, flu-like illness, and in severe cases, seizures, coma and death.

Falciparum malaria is a common parasitic infection of the CNS, and is the one of the most important parasitic disease of humans (Hay, Guerra, Tatem, Noor, & Snow, 2004; WHO, 2005). Infection with *P. falciparum* ranges from asymptomatic infection to fatal disease. There is a difference in the clinical presentation between people living in endemic areas and non-immunes such as travellers. The former are exposed to repeated malaria infections from birth and generally attain some immunity to disease in the first decade of life (Newton & Warrell, 1998). In endemic areas, between 20 and 70% of the population have parasites detected in their blood but most do not appear to have symptoms of malaria (Snow, Craig, Newton, & Sketketee, 2003). In these areas, children under 5 years old bear the brunt of severe disease and death, although pregnant women are also susceptible. Individuals are infected many times before immunity is acquired.

People living in non-malarial zones are highly susceptible to the disease. The outcome of infection depends upon factors such as the use of chemoprophylaxis, exposure to mosquito bites and the genotype of the individual. The CNS complications of severe malaria include seizures, hallucinations, and psychosis, with cerebral malaria (CM) the most serious complication.

In endemic areas, severe malaria is defined as children with asexual forms of *P. falciparum* parasites in peripheral blood and impaired consciousness (Blantyre coma (Molyneux, Taylor, Wirima, & Borgstein, 1989) score of ≤ 4) or respiratory distress (Marsh et al., 1995). CM is defined as the presence of a peripheral asexual parasitaemia, inability to localise a painful stimulus (Blantyre coma score of ≤ 2)

and the exclusion of other encephalopathies (Newton, Hien, & White, 2000). Severe falciparum malaria causes neurocognitive impairment (Carter, Neville, & Newton, 2003; Holding et al., 1999; Kihara, Carter, & Newton, 2006; Newton, 2001).

1.4.2. *Epidemiology*

Plasmodium falciparum infects up to 500 million people worldwide and is estimated to cause 2.7 million deaths every year (WHO, 2000). It appears to have a propensity for the CNS (Newton & Krishna, 1998) and over 85% of cases of falciparum malaria occur in sub-Saharan Africa, most commonly in children under five years old (Snow, Guerra, Noor, Myint, & Hay, 2005; WHO, 2002b). Studies have shown that 11% of children with CM, particularly those with hypoglycaemia, multiple seizures and deep coma, have neurological deficits on discharge from hospital (Brewster, Kwiatkowski, & White, 1990; Crawley et al., 1996; Meremikwu, Asindi, & Ezedinachi, 1997; Molyneux et al., 1989; Newton & Krishna, 1998; Olumese, Gbadegesin, Adeyemo, Brown, & Walker, 1999). Over 50% of children recover from these deficits in 18 months. These studies, however, concentrated on the more overt neurological deficits. Few studies have been carried out to determine more subtle impairments such as cognitive difficulties.

1.5. Falciparum malaria and cognitive impairment: A systematic review

1.5.1. Introduction

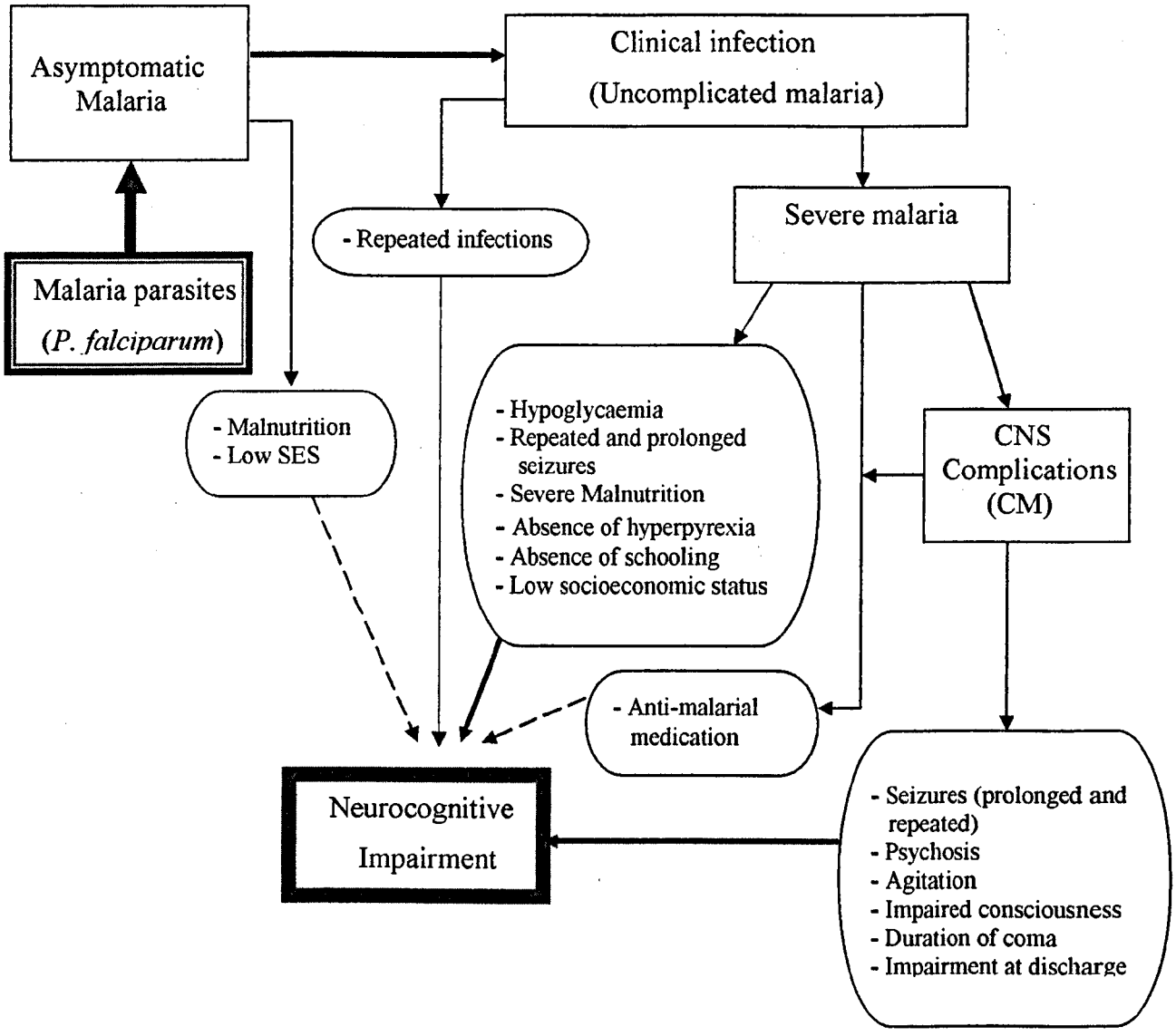
A number of studies have examined the effects of falciparum malaria on cognition but have not been reviewed. I have hereby systematically reviewed all the studies that had been carried out and published from 1966 to Dec 2005.

Neurocognitive impairment following malarial disease or parasitisation are important since this may affect the future cognitive development of children (Boivin, 2002; Carter, Mung'ala-Odera et al., 2005; Carter, Neville et al., 2003; Dugbartey, Spellacy, & Dugbartey, 1998; Holding et al., 1999; Holding et al., 2004; Muntendam, Jaffar, Bleichrodt, & Hensbroek, 1996). In addition to severe disease, various risk factors have been identified including multiple seizures, depth of coma, hypoglycaemia (Holding et al., 1999; Holding et al., 2004), multiple infections (Fernando, Gunawardena et al., 2003), schooling, multiple clinical abnormalities or complications at discharge (Holding et al., 2004), malnutrition, and socioeconomic status (Boivin, 2002; Boivin et al., 1993). The exact path that leads to cognitive impairment is obscure and is a result of many potentially interacting factors. I have, however, used available evidence to construct a simplified sketch of the probable pathways of neurocognitive impairment (Figure 1.1).

I conducted a systematic review of literature of the effects of *P. falciparum* on cognition to answer the following research questions:

1. What is the relationship between *P. falciparum* and cognitive function?
2. Are there any differences in neurocognitive impairments between children and adults?
3. Could the patterns of impairment provide insight into the mechanisms of brain damage caused by falciparum malaria?

Figure 1.1: Probable pathways of neurocognitive impairment post *P. falciparum*



Key:

- Strong evidence
- Moderate evidence
- Weak evidence
- Weakest evidence
- hypothesized but not established
- - Risk factors

1.5.2 Review methods

a) Data sources and search strategy

I carried out literature searches using three databases: MEDLINE® (1966 – Dec 2005), EMBASE® (1980 – Dec 2005) and PsycINFO (1887 – Dec 2005) using a combined text word and MESH or subject heading to identify relevant papers. Additional articles were identified by hand searching the bibliographies of these papers. In addition, relevant doctoral theses were reviewed.

The strategy was developed by breaking the review question into its elemental facets, as recommended by the National Health Service Centre for Reviews and Dissemination (Khan, Popay, & Kleijnen, 2001). These facets include exposure, outcome, population, keywords and publication language. Publication language was left open to ascertain how many studies were available in languages other than English.

b) *Study Selection*

The on-line abstracts of studies identified from the database search were reviewed and reprints of potentially eligible studies obtained. Studies meeting the following criteria were chosen for more detailed review:

1. The use of tests of cognitive function standardized for the population and/or appropriate controls.
2. Age of subjects, with at least a differentiation between children and adults.
3. In studies of severe falciparum malaria, follow-up at least 6 months after exposure to disease to exclude transient impairments and include later-developing deficits.

c) *Data extraction*

The elements of cognition to be examined in this review were divided into five categories, namely attention, memory, language, visual-spatial skills, and reasoning (executive function) (Croisile, 2004).

1.5.3. Results

The electronic search produced 34 references. A review of the abstracts showed that 15 were potentially relevant, all English language reports, despite the fact that I had not limited the language of publication. A hand search of the bibliographies identified a further 9 papers. The full text of each of the 24 papers was evaluated and 10 were excluded because the studies did not meet the review criteria: described effects of anti-malarial drug use on cognition (n=3), were review articles (n=2), did not include cognitive tests (n=2), lacked proper controls (n=1), had reported results from the same study (n=1) or did not have at least 6 months follow-up (n=1). In addition, four theses that met the inclusion criteria were identified, one of which contained two studies that were still unpublished. In total 16 studies were reviewed.

1.5.4. Overview

a) *Results of literature extraction:*

Eight (44%) of the studies that fulfilled the inclusion criteria evaluated impairments following CM, four (22%) investigated the effects of severe malaria (which included some cases of CM), five (27%) studies reported the effects of non-severe malaria, and one (5%) examined cognitive performance in participants with asymptomatic parasitaemia.

Fifteen (83%) of the studies were carried out on children and the remainder were on adults (i.e. no study examined both children and adults). Eleven (61%) of the studies were performed in Africa, five (27%) in Asia and two (10%) in North America. Seventeen (94%) of the studies had a comparison group and one (5 %) (Grote et al, 1997) used standardized tests only without a comparison group.

I have summarized the results of this literature search according to different infection levels of *P. falciparum* and its reported effect on cognition starting from the asymptomatic infections to the effects following CM.

b) *Effect of parasitaemia on cognitive function*

I identified only one study that fulfilled the criteria and that examined the effect of asymptomatic parasitaemia on performance of cognitive tasks (Al Serouri, Grantham-McGregor, Greenwood, & Costello, 2000). In a study of Yemeni children, 445 boys with asymptomatic parasitaemic boys were compared with 142 non-parasitaemic boys matched for age and schooling status (Al Serouri et al., 2000). Two weeks later, 150 children who remained parasitaemic and 150 children who were no longer parasitaemic were assessed using a battery of cognitive tests. Impairment was defined as 2 standard deviations (SD) below the control mean. The investigators found no significant differences in attention, executive functions and language scores between those who became non-parasitaemic and those who remained parasitaemic, although those with parasitaemia had impaired fine motor skills.

c) *Cognitive function during falciparum illness*

Fernando (2001) reported that the academic performance of 293 children experiencing an acute attack of uncomplicated malaria was poorer than that of 162 children experiencing an acute attack of non-malarial fever or that of 305 healthy controls. Impairment was defined as 2 SD lower than control mean. Although the performance of the children with malaria improved two weeks after treatment, it was still significantly lower than that of the healthy controls. This study was unable to examine whether poor performance was due to malaria *per se*, or the absenteeism from school caused by having an illness.

The same investigators also conducted a randomized double-blind placebo controlled study of antimalarial prophylaxis with school-age Sri Lankan children, in which they measured academic performance using school tests of language and mathematics (Fernando, 2001). Two hundred and ninety five children received chloroquine while another 292 children received placebo. They found an improvement in academic performance in those on prophylaxis for a nine month period. However, the placebo group also had significantly higher rates of absenteeism. A multivariate model identified absenteeism due to malaria and chloroquine prophylaxis as significant predictors of school performance.

d) *Cognitive function post-severe malaria*

I identified three studies investigating the effects of severe malaria on cognitive function following exposure to severe malaria, which also included children who fulfilled the definition of CM (Carter, Mung'ala-Odera et al., 2005; Carter, Neville

et al., 2003; Holding et al., 1999; Holding et al., 2004). Each of these studies investigated Kenyan children from the same area.

Holding and colleagues (1999) compared 87 children with a history of severe malaria with impaired consciousness (defined as a Blantyre score of ≤ 4 (Molyneux et al., 1989)) with community controls on tasks measuring information processing, language and behaviour based on the K-ABC (Kaufman & Kaufman, 1993) and other locally-established tests. Impairment was defined as ≥ 2 SD below the mean of the control group. The results on information processing skills showed no impairment in performance for the majority of children, although a significantly greater number of cases showed impaired performance. There were deficits in measures of language and attention/planning in which significant differences were found between cases and controls.

Holding and her colleagues presented a re-analysis of their previously reported data (Holding et al., 2004). In the re-analysis, children with a history of severe malaria were divided into medium- and high-risk groups based on the severity of the disease; these factors included laboratory findings (haemoglobin, parasite count and blood glucose), length and depth of coma, number of seizures, and respiratory distress Seventeen children were considered high-risk and the remainder, medium-risk (Holding et al., 2004). These children were then compared to controls (low-risk group) matched for age, gender and socioeconomic status. Impairment was defined as > 2 SD below the mean for the low-risk group. The results revealed that unschooled high-risk children performed significantly more poorly than unschooled children in the other two groups.

Carter and colleagues compared 25 children previously admitted to hospital with CM or severe malaria (defined as malaria prostration, multiple seizures or severe anaemia) and 27 children unexposed to either condition and found that language performance was poorer in the children exposed to either CM or severe malaria up to six years post-insult (Carter, Neville et al., 2003). Assessments of comprehension, syntax, lexical semantics, higher-level language abilities, pragmatics and phonology were administered to each child. The investigators found that children with a history of CM or severe malaria performed significantly poorer on tests of comprehension, syntax and lexical semantics compared to the unexposed group. There was no evidence of a difference between the scores of children who had suffered CM and those who had severe malaria, although this may have been due to the small numbers.

Another study by Carter and her colleagues compared 156 children exposed to malaria with complicated seizures (M/S) (defined as >2 seizures within 24 hours or focal or prolonged for >30 minutes but without coma) with children unexposed to severe malaria (Carter, Mung'ala-Odera et al., 2005; Carter, Ross et al., 2005). The exposed children belonged to a cohort that was assessed after a period of over two years post discharge. The results indicate that M/S is associated with significantly increased odds of impairment (defined as 2 SD or less than 2% of the normative data from the controls) in two aspects of speech and language (pragmatics and phonology) relative to unexposed children (Carter, Mung'ala-Odera et al., 2005). There was no evidence of a difference in performance on tests of memory, attention or other aspects of language.

e) *Cognitive function post-cerebral malaria*

Many studies have reported neurocognitive deficits associated with severe malaria, particularly CM.

In a Ghanaian study, 20 children with a history of CM (defined using the WHO criteria) aged between 7-16 years old were compared with 20 controls matched for age, sex and educational level, and assessed with a standardized neuropsychological battery (Dugbartey, 1995b; Dugbartey & Spellacy, 1997; Dugbartey, Spellacy et al., 1998). Children with a history of CM performed significantly poorer relative to controls in bimanual tactile discrimination, accuracy of visual scanning, visual memory, perceptual abstraction and rule learning skill, right ear auditory information processing, and dominant-hand motor speed. The study found no significant differences between those with a history of CM and controls in non-verbal reasoning, visual-spatial processing, auditory attention and verbal fluency (Dugbartey, 1995b).

In Senegal, 29 children aged between 5-12 years old with a history of CM (defined using WHO criteria but with coma duration adjusted to 12 hours) were compared with 29 children with 'mild-malaria' matched for age and education. Since children with no history of malaria are uncommon, the controls were referred to as 'mild malaria' group. They had no history of coma, seizures, head injury or trauma. Those with a history of CM performed significantly poorer on the simultaneous processing (spatial memory, photo series), mental processing, and sequential processing (hand movements, word order) tasks of the K-ABC and the attention

capacity task from Test of Attention Variables (TOVA) than those with mild malaria (Boivin, 2002). The study found significant correlation between coma duration and attention capacity for the CM group emphasizing the importance coma duration on outcome.

In Kenya, 152 children aged between 6-9 years old previously exposed to CM (defined as a Blantyre coma score of ≤2 for 4 or more hours, a peripheral parasitaemia and the exclusion of other causes of encephalopathy) (Newton et al., 2000) were compared to 179 children unexposed to severe malaria (Carter, Mung'ala-Odera et al., 2005; Carter, Ross et al., 2005). Impairment was defined as 2 SD lower than the mean. The performance of children previously exposed to CM was poorer than unexposed children on all the cognitive assessments administered: speech and language battery, attention, memory and non-verbal functioning. Significant differences were found on tests of higher-level language abilities, lexical semantics, pragmatics and non-verbal functioning (construction task).

There was one study that did not detect any differences between CM (defined as a Blantyre coma score of ≤2) and controls. This case-control study focused on the measurement of non-verbal functioning in 36 pairs of Gambian children who were discharged from hospital without any neurological deficits. Impairment was defined as 1 SD lower than the control group mean. The results showed no significant impairments in children after an average follow-up of 3.4 years (Muntendam et al., 1996).

f) *Effect of Age at exposure on cognitive function*

Age may influence recovery from neurocognitive impairment. We found no published literature describing the effects of age on the cognitive performance of both children and adults following falciparum malaria. Three studies documented the effects of falciparum malaria on the cognitive performance of adults (Dugbartey, Dugbartey, & Apedo, 1998; Grote, Pierre-Louis, & Durward, 1997; Richardson, Varney, Roberts, Springer, & Wood, 1997). Kastl and his colleagues (1968) had reported that CM patients made complete recovery with no residual deficits. A study on Ghanaian adults with non-severe malaria showed similar negative findings (Dugbartey, Dugbartey et al., 1998). The other studies have found neurocognitive sequelae many years post-CM (Dugbartey, Dugbartey et al., 1998; Grote et al., 1997; Richardson et al., 1997). However, none of these studies tested all the five facets of cognition making it difficult to identify patterns of neurocognitive impairment.

Children exposed to acute falciparum malaria may have deficits in the cognitive dimensions studied, like attention (Boivin, 2002; Holding et al., 1999; Holding et al., 2004), memory (Boivin, 2002; Dugbartey, 1995b; Dugbartey, Spellacy et al., 1998; Holding et al., 2004), language (Carter, Mung'ala-Odera et al., 2005; Carter, Murira, Ross, Mung'ala-Odera, & Newton, 2003; Fernando, Wickremasinghe, Mendis, & Wickremasinghe, 2003; Holding et al., 1999), visual-spatial skills (Boivin, 2002; Dugbartey & Spellacy, 1997; Fernando, Wickremasinghe et al., 2003) and executive function (Carter, Mung'ala-Odera et al., 2005; Dugbartey, 1995a; Dugbartey, Spellacy et al., 1998; Holding et al., 2004).

1.5.5. Discussion

a) *Summary of the main results*

These results suggest that falciparum malaria affects neurocognitive performance both in the short- and long-term. The hypothesis that falciparum malaria affects a specific brain location appears unlikely given the wide variety of neuropsychological outcomes post infection. However, there are still few data on which to assess the degree and extent of the impairments.

In children (Table 1.1), most studies reported that increased severity of malaria is associated with greater degrees of neurological and cognitive impairment (Boivin, 2002; Carter, Mung'ala-Odera et al., 2005; Carter, Neville et al., 2003; Dugbartey, 1995a; Dugbartey & Spellacy, 1997; Dugbartey, Spellacy et al., 1998; Holding et al., 1999; Holding et al., 2004), while only one suggests that there is complete recovery with no significant reduction in cognitive abilities (Muntendam et al., 1996). This discrepancy could be explained by small sample size of the latter study, and possibly did not have enough power to show the effect. Further, the test battery may have lacked sensitivity to detect subtle differences. The exclusion of children with neurological deficits at discharge could have excluded the very children who are most vulnerable to neurocognitive sequelae.

In adults (Table 1.1), the results are more difficult to interpret. First, there are few studies, then the definition of CM was not based on any standard criterion and finally they have methodological differences. However, cognitive deficits were found in attention, memory and language while none were present in visual-spatial skills and executive functions.

Some studies conclude that cognitive impairment also occurs after non-severe malarial disease (Fernando, 2001; Fernando, Gunawardena et al., 2003; Fernando, Wickremasinghe et al., 2003). The Sri Lankan study (Fernando, 2001) shows that the children who had non-severe malarial infection still performed significantly poorer in scholastic tasks two weeks post-acute infection compared to controls.

Table 1.1: Studies examining cognitive function following falciparum malaria

	Country	Population (years)	clinical description	Criteria	No. Cases	Controls	Follow-up length	Study Design	Attention	Memory	Language	visual spatial skills	executive functions
Carter JA et al (2005)	Kenya	6 - 10 yrs	CM	Blantyre coma score of ≤ 2	152	179	> 2 yrs	Cohort Study	Not significant	Not significant	performed significantly poorer *(P < 0.05)*	NT	performed significantly poorer *(P < 0.05)*
Carter JA et al (2005)	Kenya	6 - 10 yrs	M/S	Blantyre coma score of ≤ 2	156	179	> 2 yrs	Cohort Study	Not significant	Not significant	performed significantly poorer *(P < 0.05)*	NT	Not significant
Carter JA et al (2003)	Kenya	8 - 9 yrs	CM & Severe Malaria	Blantyre coma score of < 2 (W.H.O criteria)	25	27	> 2 yrs	Cohort Study	NT	NT	performed significantly poorer *(p =0.004)*	NT	NT
Fernando SD et al (2003)	Sri Lanka	5 - 6 yrs	Malaria	N/A	171	154	1 yr	Cross-sectional	NT	NT	borderline performance *(P = 0.093)*	Not significant	NT
Fernando SD et al (2003)	Sri Lanka	6 - 14 yrs	Malaria	N/A	385	213	1-6 yrs	Prospective	NT	NT	performed significantly poorer *(P < 0.001)*	performed significantly poorer *(P < 0.001)*	NT
Boivin MJ (2002)	Senegal	5 - 12 yrs	CM	W.H.O. criteria for CM	29	29	avg 3.4 yrs	Matched case-control	performed significantly poorer (p < 0.05)	performed significantly poorer *(P < 0.05)*	NT	performed significantly poorer *(P < 0.05)*	borderline significance *(P = 0.07)*
Fernando SD (2001b)	Sri Lanka	6 - 12 yrs	Malaria	N/A	295	292	N/A	Randomized - control study	NT	NT	performed significantly poorer *(P < 0.001)*		NT
Fernando SD (2001a)	Sri Lanka	5 - 12 yrs	Malaria	N/A	343	305	1-2 yrs	Prospective	NT	NT	performed significantly poorer *(P < 0.001)*	NT	NT
Al Serouri AW et al (2000	Yemen	11 - 13 yrs	Asymptomatic	N/A	445	142	N/A	Matched case-control	Not significant	Not significant	NT	NT	Not significant

Table 1.1: Studies examining cognitive function following falciparum malaria

First Author	Country	Population (years)	clinical description	Criteria	No. Cases	controls	Follow-up length	Study Design	Attention	Memory	Language	visual spatial skills	executive functions
Holding PA et al (1999)	Kenya	6 - 10 yrs	Severe Malaria	Blantyre coma score of ? 4	87	87	3.5 - 6 yrs	Matched case-control	performed significantly poorer ($p < 0.05$)	Not significant	performed significantly poorer *($P = 0.02$)*	Not significant	borderline performance *($P = 0.06$)*
Dugbartey AT et al (1998)	Ghana	7 - 16 yrs	CM	W.H.O. criteria for CM	20	20	avg 3.9 yrs	Matched case-control	Not significant	performed significantly poorer ($p < 0.01$)	Not significant	Not significant	performed significantly poorer
Mutendam AH et al (1996)	Gambia	5 - 9 yrs	CM	Blantyre coma score of ? 2	36	36	avg 3.4 yrs	Matched case-control	NT	Not significant	Not significant	Not significant	Not significant
Dugbartey AT et al (1997)	Ghana	7 - 16 yrs	CM	W.H.O. criteria for CM	20	20	avg 3.9 yrs	Matched case-control	NT	NT	NT	performed significantly poorer *($P < 0.01$)*	NT
Adults													
Dugbartey AT et al (1998)	Ghana	18 - 68 yrs	Malaria	N/A	142	30	> 1 yr	case - control	Not significant	NT	Not significant	NT	Not significant
Grote CL et al (1997)	USA	38 yrs	CM	N/A	1	0	10 yrs	Case study	No Impairment	delayed memory impairment	NT	No impairment	NT
Richardson ED et al (1997)	USA	35 - 55 yrs	CM	N/A	40	40	> 20 yrs	Retrospective	performed significantly poorer ($p < 0.05$)	performed significantly poorer *($P < 0.001$)*	performed significantly poorer *($P < 0.001$)*	NT	NT

NT, means 'not tested'; CM, cerebral malaria; M/S, malaria with seizures

Further findings have also pointed to the possibility that repeated non-severe malaria infections have a significant negative effect on cognitive performance, manifesting as impairment in school performance (Fernando, 2001; Fernando, Gunawardena et al., 2003). The trial performed in Sri Lanka aimed to differentiate the effects of malarial infection from school absence on school performance. However, the group that received the placebo was absent from school significantly more times than the group receiving chloroquine. It remains difficult to ascertain whether it was the malaria *per se* or influence of other risk factors that affected academic performance.

Studies that examined asymptomatic children did not detect any appreciable differences between when they were parasitaemic and when non-parasitaemic (Al Serouri et al., 2000; Boivin et al., 1993), perhaps because of very low parasitaemia. Furthermore, the two-week duration in the Yemeni study, used on follow-up may be too short to detect appreciable differences.

b) *Limitations of the studies*

One of the difficulties of attributing effects of *P. falciparum*, particularly in malaria endemic areas, is the exclusion of other causes. In a recent study of Malawian children who fulfilled the WHO criteria of CM, 23% were found to have other causes of death at post-mortem (T. E. Taylor et al., 2004). The lack of specificity of the diagnosis affects all the studies of severe malaria reported from Africa and may influence the interpretation of results of these studies.

There is no universally accepted system for defining impairment on non-standardized neuropsychological assessments. Many studies used different definitions for impairment, making cross-study comparisons difficult. Further, studies had different criteria for defining their cases and controls complicating the comparison process even more. A variety of risk factors were identified in some studies e.g. effects of duration of coma, hypoglycaemia, socioeconomic status, malnutrition but the same factors were not examined in all studies. Also, in endemic areas, it is difficult to find children who have never had malarial illness. Hence it is possible that the comparative groups in the reported studies may have compromised cognitive abilities just like the exposed children. Some studies had very small sample sizes (Dugbartey, Spellacy et al., 1998; Muntendam et al., 1996), which may have affected their results. It is also possible that malaria parasitaemia may affect cognitive function but the instruments may not be sensitive enough to detect subtle deficits. In the Fernando, Gunawardena *et al.* (2003) study, they did not account for age at entry of school, the fact that 13% children did not have pre-school education and depended on parental/guardian reports for frequency of malarial episodes.

Several studies (Dugbartey, Spellacy et al., 1998; Muntendam et al., 1996) used tests that were developed in other cultures and their psychometric properties in local populations were not reported. The use of adapted neuropsychological or cognitive assessment tests, rather than tests developed for local populations, makes interpretation difficult since no assumptions can be made that the same neuropsychological/cognitive function is being measured (Greenfield, 1997). Other studies did not adequately define the expected outcome measures. The Sri Lankan

studies (Fernando, 2001; Fernando, Gunawardena et al., 2003; Fernando, Wickremasinghe et al., 2003) used mathematics and language tests as measures of impairment. These tests may not be an appropriate assessment of cognition and could not identify possible brain regions that are affected by malaria infection.

c) Mechanisms of neurocognitive impairment

The exact mechanisms by which *P. falciparum* causes neurocognitive impairment remain unknown. Malaria infection can indirectly affect cognition through nutrition, school attendance or psychosocial development, all of which may contribute to delay in or sub-optimal cognitive development [(Holding & Kitsao-wekulo, 2003); Figure 1.1]. For example, children with malaria are reported to have poorer nutritional status than non-malarial children (Fernando, Wickremasinghe et al., 2003; McGregor, 1988; Shiff et al., 1996). Although malnutrition is associated with poorer cognitive development (Bryan et al., 2004), it is unclear if this is a direct effect of malaria itself. The impact of malaria-induced anaemia remains unstudied, but iron deficiency anaemia which often occurs in children with malaria impairs school performance (Grantham-McGregor & Ani, 2001; Lozoff, Jimenez, & Wolf, 1991). There is strong evidence that CM causes neurocognitive impairment as a result of direct brain damage. Specific impairments such as hippocampal dysfunction and damage to sub-cortical white matter may lead to impairments of memory and language function (Grote et al., 1997; Richardson et al., 1997; Varney, Roberts, Springer, Connell, & Wood, 1997). The study by Richardson and her colleagues (1994) on a dichotic listening task by Vietnam veterans with a history of CM showed that adults may have inefficiencies in processing auditory information many years post infection. This finding further

points to the possibility that CM affects the brain's white matter structure. Also, it has been suggested that neurotoxins released from infected red blood cells may damage the cortical areas of the brain, affecting cognitive performance (Fernando, 2001).

I identified only two published studies that performed tests on the whole spectrum of cognitive domains under review (Dugbartey, Spellacy et al., 1998; Holding et al., 1999; Holding et al., 2004). This makes it difficult to draw firm conclusions about patterns of impairment. Further, in all the studies reviewed, there was no particular facet of cognition that was consistently impaired in either children or adults following exposure to falciparum malaria.

1.5.6. Conclusion

These results indicate that *P. falciparum* is associated with neurocognitive impairment both during infection and following severe disease. There are many risk factors other than malarial disease that seem to affect cognitive outcome, and these need to be studied in a more systematic manner. There may be a difference in impairment patterns following severe disease in children and adults, although this needs to be explored further. The age of exposure to severe disease may prove an important variable, as well as the total number repeated infections. The impairment appears diffuse, with children generally having cognitive impairment in most cognitive domains, while adults seem relatively unaffected in visual-spatial skills and executive function. Further research is required to determine the damaging effects of *P. falciparum* infection: as it infects over 500 million people per year, it is potentially one of the most important causes of cognitive impairment worldwide.

1.6. Summary

Insults to the brain resulting from acute illness could have far reaching negative cognitive outcomes. The review of extant literature has shown that ABM, HIV infection and severe falciparum malaria can lead to cognitive impairment. The deficits are sometimes subtle but may adversely affect the child's social and educational functioning. There is need to determine the cognitive effects of exposure to these brain insults in SSA as there are little data.

CHAPTER 2

Cognitive neuropsychology and child assessment

2.1. Introduction

Cognitive neuropsychology is a branch of neuropsychology that aims to understand how the structure and function of the brain relates to specific psychological processes. It places a particular emphasis on studying the cognitive effects of brain injury or neurological illness with a view to inferring models of normal cognitive functioning. Children's brains are particularly susceptible to insults, and cognitive neuropsychology offers a means to estimating the extent of damage, level of impairment and potential for recovery and long-term functioning of the individual. Damage to the brain often has potentially detrimental consequences for the individual. It could affect those basic skills and abilities which are necessary for everyday life. Neuropsychological assessments in children aim to obtain information about the integrity of the CNS and the child's abilities (H. G. Taylor & Schatschneider, 1992).

2.2. The normal brain development

Childhood is a period of potential growth of the brain, which allows the child to acquire skills to function as an independent person. The brain volume quadruples between birth and adulthood due to an increase in the size of neurons, and subsequent increase in number of supporting cells (glia), development of fatty tissues around the neuron (myelination) and synapses (Johnson, 1997; Shonkoff &

Phillips, 2000). The myelination process increases the efficiency of information processing and is thought to play a critical role in cognitive development.

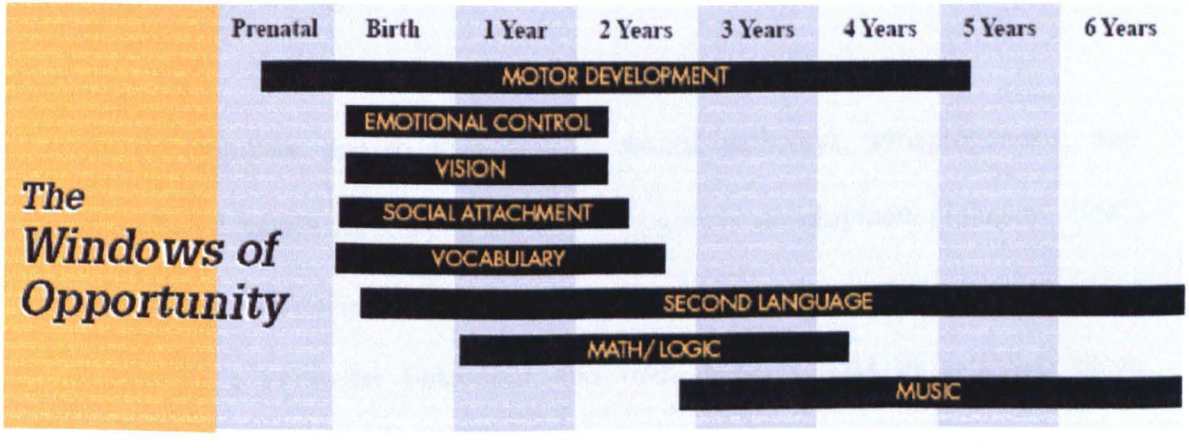
Brain functions are expressed through activity of neural circuits that are formed throughout the foetal period and throughout the life by the formation of synapses in a process that is called synaptogenesis. There is evidence in many parts of the nervous system that the stability and strength of these synapses are largely determined by activity i.e. firing of these connections (Shonkoff & Phillips, 2000). Connections between neurons are formed as the growing child experiences the surrounding world and forms attachments to parents, family members and other caregivers.

Developmental research shows that post natal brain development and learning depends critically and continually on the interactions between nature (an individual's genetic endowment) and nurture (the nutrition, surroundings, care, stimulation, and teaching that are provided or withheld) (Johnson, 1997; Shonkoff & Phillips, 2000; Shore, 1997). The impact of environmental factors on the young child's brain development can be large and specific, not merely influencing the general direction of development, but also affecting how the intricate circuitry of the human brain is "wired" (Shonkoff & Phillips, 2000). There are diverse views of the effect of the environment on the developing brain. Some propose an interactive model in which presumes that the nurture variables remain independent and unaltered in the course of interaction (Lerner, 1980; Sameroff, 1975, 1998) while others propose a transactional model that emphasizes reciprocal change in both nurture and nature variables (Goldberg, 1978; Sameroff & Mackenzie, 2003). The

interactive model assumes that environments have their effects either positive or negative, independent of the characteristics of the individuals who experience them while in the transactional model, even the characteristics of the person is also crucial. The interactive model seems to suggest that the locus of control is outside the organism (MacDonald, 1986). In view of the variability in the ultimate outcome, the use of "normal" brain development in humans is interpreted with caution.

Research has also showed that there are "developmental windows of opportunity" or "critical periods" for different functional brain development (Brainfacts, 2005). Critical periods in brain development accommodate the development of specific skills during which the brain is active in forming connections for specific abilities (Figure 2.1). While critical periods are prime times for the development of specific neural synapses, skills can still be learned after a window of opportunity has closed, but with greater time and effort. It is during these critical periods that lack of stimulation or negative experiences can have the most impact.

Figure 2.1: Critical periods for opportunity (Adapted from *Re-thinking the brain, 1996)*



Brain malformations may result from exogenous and endogenous causes. Exogenous causes could either be biological or psychosocial. Early biological insults such as malnutrition, infectious diseases, exposure to radioactivity, viral infections, chemical substances, medications, ischemia or prematurity could hinder optimal brain development. Psychosocial risks such as stress, poverty and family violence are also thought to result in impaired brain development (Johnson, 2001; Shonkoff & Phillips, 2000). Endogenous causes are genetic in nature. The foetal brain and the young infant's brain are especially vulnerable to damage from toxins and malnutrition, although cognitive impairments may not become apparent until learning disabilities appear many years later (Shore, 1997).

2.3. Cognition and brain development

The term *cognition* is derived from the Latin word "*cognoscere*" which means "to know". It generally refers to a faculty for information processing. Cognition or cognitive processes can be natural and artificial, conscious and not conscious. It is used to refer to the mental functions, mental processes and states of intelligence. The sort of mental processes described as cognitive or cognitive processes include memory, attention, perception, action, problem solving and mental imagery (Spreen, Risser, & Edgell, 1995).

Brain development due to formation of neural pathways, synaptogenesis, and myelination does not correspond directly to cognitive development (Johnson, 1997; Shore, 1997). Neuropsychologists hypothesize that a particular part of the brain is involved in a particular behaviour and then develop tests to establish these hypothesis. The historical case of H.M, who suffered from medically intractable

epilepsy, is an example of this approach. The seizures in H.M originated from the mesial temporal lobe, so both his temporal lobes were surgically removed to save him from potentially life-threatening complications. The surgery brought his seizures under control, but H.M was left without the ability to form new memories. The hypothesis confirmed in this case was the critical role of the hippocampus in the formation of memory (Nelson & Bloom, 1997). The development of high density event related potentials and functional magnetic resonance imaging (fMRI) has renewed efforts to explore the interface between brain development and cognitive development. Another useful tool in linking brain development to behaviour is the use of "marker tasks". This method involves the use of specific behavioural tasks which have been related to one or more brain regions in adult human by neuropsychologists or through brain-imaging studies. However, the use of markers in infants and young children may not be feasible especially if they require verbal instruction or output. Also, infants and young children have a short attention span and cannot cooperate for long. New methods based on "stimuli preference" and "habituation" have been useful for these young populations (Johnson, 1997; Nelson, 1994).

2.4. Standardized neurocognitive assessment

Standardized neuropsychological testing is used to assess a wide range of abilities, including attention, memory, problem solving, language skills and intellectual functioning. It is the process of determining a patient's cognitive strengths and weaknesses through qualitative (approach to tasks and observed behaviour) and quantitative (standardized and scaled measures) approaches (Costa, Azambuja, Portuguez, & Costa, 2004). Test scores are interpreted on the basis of normative

data and expected level of performance for a given individual based upon their educational/occupational level.

Cognitive difficulties are a recognized consequence of brain insults. Infectious diseases can cause widespread damage including impaired verbal learning and memory, loss of attention/concentration, impaired speed of thinking and executive functions (e.g. concept formation and abstraction). Appropriate tools (neuropsychological tests and developmental assessment scales) are necessary to show the major gains during development and aim at determining the child's specific level of development. The aim of these tools is the early detection of developmental and learning disabilities (Costa et al., 2004).

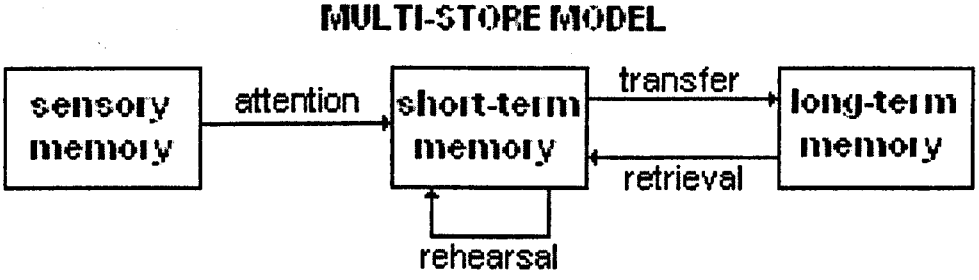
The three domains often assessed in standardized neurocognitive assessment are memory, attention and executive function as they help establish proper development of higher cortical functions.

2.4.1 Memory

Memory refers to an organism's ability to store, retain, and subsequently recall information. Poor memory function can impair a child's opportunities to learn and can lead to learning disability (Gathercole, 1998). Researchers distinguish several types of memory systems that can function relatively independently on the basis of duration of memory retention, and identify three distinct types of memory: sensory memory, short-term memory and long-term memory (Figure 2.2). A further distinction within the long-term memory is between memory of events (episodic)

and the knowledge of factual information (semantic or autobiographical) (Tulving, 1972).

Figure 2.2: The memory process [Adapted from (Atkinson & Shiffrin, 1968)]



2.4.1.1. Short-term memory

This is the memory of events that occurred in the very recent past whereby the recall of learned information is measured in terms of seconds or minutes as opposed to hours or days. (Baddeley & Hitch, 1974) proposed a model comprising of the central executive, the phonological loop and the visualspatial sketchpad to account for formation of short-term memories or working memory. The phonological loop stores limited sound-based material, while the visualspatial sketchpad stores a limited amount of visual and spatial properties of information. The central executive acts as a coordinator of these two domains, helps in the retrieval of information from the long-term memory, logical reasoning and mental arithmetic (Baddeley, 1986).

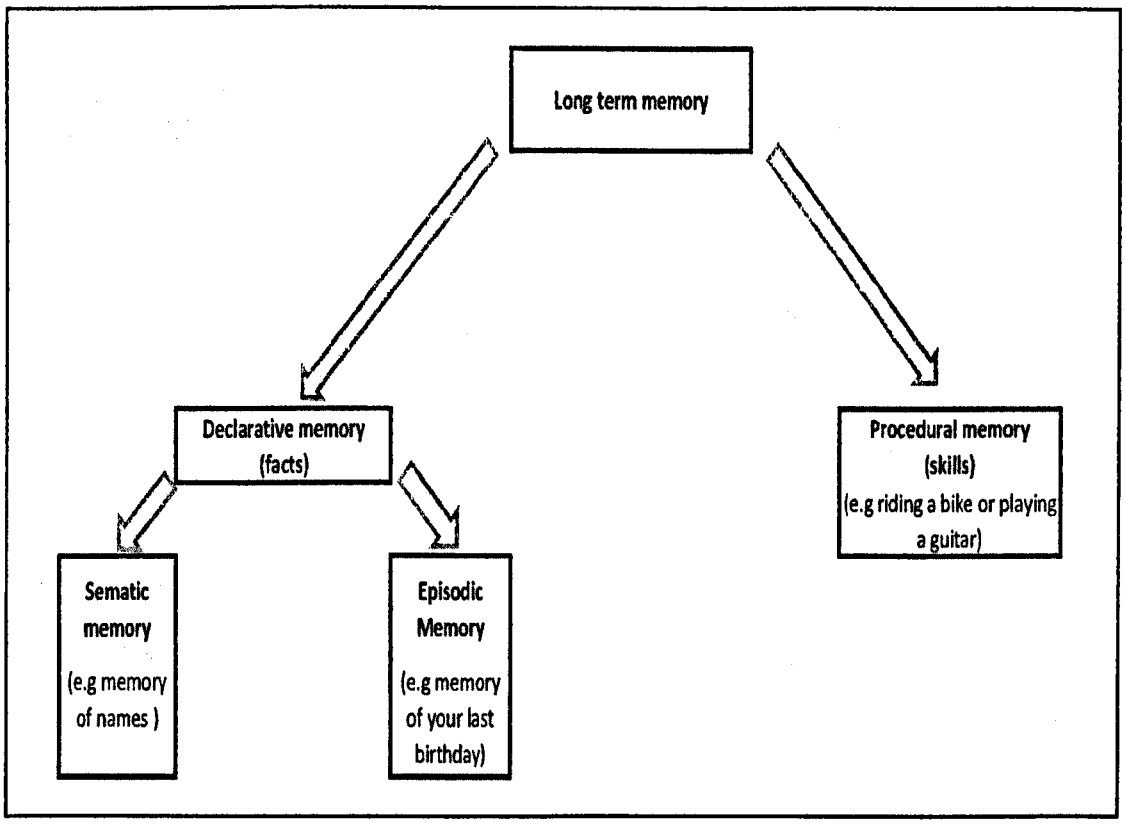
While the understanding of brain regions that mediate the three domains are somewhat incomplete, there is converging evidence that the phonological loop's short-term memory is mediated by the left-hemisphere's regions of the Broca's area and the prefrontal cortex and the visuospatial sketchpad is mediated by parietal and

pre-frontal areas of the right hemisphere (E. E. Smith, Jonides, & Koeppe, 1996). The working memory may be compromised by a number of neurological and psychiatric conditions that may lead to behavioural and cognitive deficits (Dash, Moore, Kobori, & Runyan, 2007).

2.4.1.2. Long-term memory

Long-term memory is typically divided up into two: declarative memory and implicit memory (or procedural memory). Declarative memory refers to memories that are consciously available. These are encoded by the hippocampus, entorhinal cortex, and perirhinal cortex, but consolidated and stored elsewhere in the cortex (Graham & Hodges, 1997; Reed & Squire, 1998; Rempel-Clower, Zola, Squire, & Amaral, 1996). The declarative memory also has two distinct memory systems; the semantic and episodic memory (Tulving, 1972) [Figure 2.3]. Semantic memory refers to knowledge of the world i.e knowledge of names or facts without recollection of when and/or where the information was learnt, while episodic memory refers to memories of specific episodes previously experienced (Gathercole, 1998). Procedural memory refers to the use of objects or movements of the body, such as how exactly to use a pencil or ride a bicycle. This type of memory is encoded and probably stored by the cerebellum and the striatum.

Figure 2.3: Sub-systems of the Long-term memory



Current studies in neuroscience strongly support the notion that while memory does not represent a singular store, it involves the formation of new neural connections or the modification of old ones. Encoding can take place in several parts of the brain thus neural connections are likely to involve various parts of the brain. There is better recollection of events if the neural connections are stronger (Schacter, Curran, Galluccio, Milberg, & Bates, 1996). Recollection of an event can occur by a stimulus to any of the parts of the brain where a neural connection for the memory occurs. If part of the brain is damaged, access to the neural data that was in that part of the brain is lost. The evidence is strong that there are distinct types and elements of memory which involve different parts of the brain, e.g., the hippocampus responsible for short-term memory (ongoing incidents of day-to-day living); the amygdala for emotional memories (Schacter et al., 1996); the Broca's area and

prefrontal cortex of the left hemisphere are associated with phonological short-term memory and the parietal regions of the right hemisphere are associated with visuospatial memories (E. E. Smith et al., 1996). Damage to the hippocampus/ medial temporal lobe is closely associated with the loss of episodic memory (Rempel-Clower et al., 1996; Vargha-Khadem et al., 1997) with relative preservation of the semantic memory.

Neuropsychological tests assess explicit memory or conscious recollection (for facts or events) as opposed to implicit (skills and procedures) traces. There are separate dimensions of memory – working (short-term), stored (long-term), verbal, spatial (visual) and learning capacity. Individuals may have impairment in one domain but not in another. It is important to test both delayed recall and recognition.

2.4.2 Attention

Attention is the cognitive process of selectively concentrating on one aspect of the environment while ignoring other things. Sohlberg and Mateer (1989) proposed a hierarchic model that is based in the recovery of attention processes of brain damaged patients after coma (Sohlberg & Mateer, 1989). Five different kinds of activities of increasing difficulty are described in the model: *Focused attention* (the ability to respond discretely to specific visual, auditory or tactile stimuli), *Sustained attention* (the ability to maintain a consistent behavioural response during continuous and repetitive activity, *Selective attention* (the capacity to maintain a behavioural or cognitive set in the face of distracting or competing stimuli. Therefore it incorporates the notion of "freedom from distractibility"), *Alternating*

attention (the capacity for mental flexibility that allows individuals to shift their focus of attention and move between tasks having different cognitive requirements) and *Divided attention* (This is the highest level of attention and it refers to the ability to respond simultaneously to multiple tasks or multiple task demands).

Many children with attention deficit hyperactive disorder (ADHD) do poorly on tests of sustained and selective attention. Sustained attention is proposed to be mediated by the reticular formation and the brain stem structures (Mirsky, 1996; Mirsky, Anthony, Duncan, Ahearn, & Kellam, 1991) with some involvement of the frontal regions (Stuss, Shallice, Alexander, & Picton, 1995). Impairments in sustained attention may potentially impact on the child's ability to acquire and integrate new skills and knowledge (Betts, McKay, Maruff, & Anderson, 2006). The involvement of the hippocampus in attention is supported by both behavioural and electrophysiological measures (Mirsky, 1987).

Neuropsychological tests of attention assess reaction time since it is assumed that the child must remain attentive to perform efficiently. Other tests of attention involve a continuous externally paced vigilance task. These tasks involve monitoring of a signal over time with an appropriate response. There are also tests that involve dual activities to test divided attention. However, there is inconsistency in the literature on the performance of children with ADHD on the various tests (Barkley, 1997; Barkley, Grodzinsky, & DuPaul, 1992).

Attention is supported by different brain regions making it vulnerable and a common sequela to brain dysfunction (Mirsky, 1987). There is need for a multi-modal approach in the investigation of attention to tap all these brain regions.

2.4.3 Executive functions

Executive functions were first described as a "central executive" (Baddeley & Hitch, 1974) and later as a dimension that deals with "how" behaviour is expressed (Lezak, 1983). Executive functions are said to represent four components; the abilities of goal formation, planning and carrying out goal-directed plans and effective performance (Jarudo & Rosselli, 2007). Different executive functions include: the ability to sustain or flexibly redirect attention, the inhibition of inappropriate behavioural or emotional responses, the planning of strategies for future behaviour, the initiation and execution of these strategies, and the ability to flexibly switch among problem-solving strategies (Luria, 1966; Spreen et al., 1995). Current research evidence suggests that executive functioning in the human brain is mediated by the prefrontal lobes of the cerebral cortex (Luria, 1973; Stuss et al., 2002). Theories of the executive system were largely driven by observations of patients who had suffered frontal lobe damage (Stuss & Benson, 1986). They exhibited disorganized actions and strategies for everyday tasks (a group of behaviours now known as dysexecutive syndrome) although they seemed to perform normally when clinical or lab based tests were used to assess more fundamental cognitive functions such as memory, learning, language and reasoning. Neuroimaging studies have demonstrated the involvement of the frontal lobes, especially the prefrontal cortex, while engaging in executive tasks but now different regions of the frontal lobe are accepted as associated with executive functions

(Stuss & Alexander, 2000; Stuss et al., 2002) as well as other sub-cortical structures and thalamic pathways (Lewis, Dove, Robbins, Barker, & Owen, 2004; Monchi, Petrides, Strafella, Worsley, & Doyon, 2006).

The neuropsychological evaluation of executive functions relies on tests that test the frontal lobe functions. Most tests are task-based and are useful in identifying capacities, processes and abilities that are impaired in patients with frontal lobe dysfunction (Jarudo & Rosselli, 2007). However there remains controversy whether these tests tap executive functions only or if other non-executive processes are tested too.

2.5. Neurocognitive assessment in Africa

In sub-Saharan Africa, there are few studies of neurocognitive development mainly because of the lack of culturally appropriate assessment tools and procedures (Holding & Kitsao-wekulo, 2003). Although children growing up in SSA are exposed to a number of potentially debilitating diseases, few studies have examined the development in children in the region following exposure to early brain insult (Mung'ala-Odera, Snow, & Newton, 2004). Cognitive tests, sensitive to early brain insults, which can be used over a wide age range and can be compared across population groups, are required for research purposes. Previous studies of the sequelae of brain insults in childhood and of the prevalence of neuropsychological impairment carried out at different locations in Africa have used cross sectional analyses of the performance on neuropsychological tests of school aged children. These studies have provided evidence of the persistent burden of disease.

In addition to paucity of data relating to cognitive impairments in children with a history of brain insults, there has been variability in the methodology used to measure outcome. This has made it difficult to draw general conclusions about their effects on cognition.

2.6. Difficulties of cognitive assessment

One of the difficulties in assessing the neurocognitive outcome of children in SSA countries is the lack of standardized cognitive tests. Most studies use tests adopted from the West and translated to a local language. There has been little investigation across cultures to determine whether the tests examine similar cognitive processes. What has been evident is that there are disparities in the performance of tests that have been adapted from Western cultures (Carter, 2002). Also, there are many interacting health and environmental factors that could lead to poor performance in cognitive tests other than brain (Boivin et al., 1993; Holding et al., 1999). A general lack of formal schooling, language limitations and limited children-adult interaction further complicates use of conventional neuropsychological tests in our setting. The use of observation tests tends to be subjective and is subject to inter-rater variability. Further, in the assessment of sequelae following acute brain insults, children of a wide range of ages need to be tested over time. However, few studies have followed up cases longitudinally to assess sequelae.

Most psychological evaluation of children in SSA has depended on the use of adapted neuropsychological tests, rather than tests developed for local populations, which may introduce a bias in their reporting and interpretation (Carter, Lees et al., 2005; Kihara et al., 2006). In view of these considerations there is need to develop

or adopt methodologies that are less dependent on the assessor, less dependent on language and can be used over a wide range of ages to assess cognition in children in a rural population.

2.7 Summary

Neuropsychological tools have the advantage of being completely non-invasive, can be used across species and can provide insight into specific behaviours but their use faces serious challenges especially in SSA. They however should not be replaced entirely but should be supplemented with other methods e.g. imaging technologies that have superior temporal and spatial resolution and free from inter-rater biases.

CHAPTER 3

Event Related Potentials

3.1. Introduction: Electroencephalography (EEG)

Electroencephalography (EEG) is a neurophysiologic measurement of the electrical activity of the brain whose discovery is credited to Richard Caton who in 1875 recorded evoked potentials to light in animals (Swartz & Goldensohn, 1998). Brain activity can be recorded simultaneously from a number of electrodes attached to the scalp. These electrodes record voltage changes that occur when a large number of cerebral neurons are activated in close proximity and in synchrony. The small electrical signals detected by the scalp electrodes are amplified thousands of times to produce distinguishable waveforms. The EEG is capable of detecting changes in electrical activity in the brain at millisecond accuracy. It has high temporal resolution. Electroencephalography has been used in cognitive assessment to measure brain function by analysing the electrical activity generated by the cortex of the brain (Byrne et al., 2001). They are frequently used in experimentation because the process is non-invasive to the research subject.

3.2. Event related potentials

Event Related Potentials (ERPs) are EEG changes that are time-locked to sensory or cognitive events and reflect the neurophysiologic processing of these events (Pfefferbaum, Roth, & Ford, 1995). While EEG patterns reflect the brain's background activity, ERPs show how the brain responds to environmental events. Currently ERPs have become frequently used research tools in the field of neuroscience and neuropsychology, because of their high temporal resolution

(ability to trace the time-course of cognitive processing) with millisecond accuracy. The usefulness of ERPs is further enhanced as they are generally non-invasive, relatively inexpensive, and do not require participants to provide motor or verbal response, yet they can detect covert responses to stimuli, such as reading, listening or differentiating. Indeed, virtually identical procedures can be used across the entire life span (Nelson, Thomas, de Haan, & Wewerka, 1998). Neuropsychological research of cognitive functioning in various populations also demonstrates ERP components as informative markers of neurodevelopment (Courchesne, 1978). ERP recordings have high temporal resolution compared to other neuroimaging techniques hence provide unique and important information about brain processing (de Haan & Thomas, 2002).

The ERP components are typically designated by their polarity, either negative (N) or positive (P), and either their sequential order (e.g. P1, P2, P3, N1, N2, etc) or peak latency measured in milliseconds (msec) after stimulus onset (e.g. "P100" is a positive peak occurring 100 msec after stimulation (Dolchin & Coles, 1988; M. J. Taylor & Baldeweg, 2002). The waves ERPs can be divided into two categories: exogenous and endogenous. Exogenous ('early' or 'sensory' or 'obligatory') components are elicited by sensory stimuli and represent brain response to the occurrence of the stimuli. They occur with the first 100-200 msec post stimulus presentation and are sensitive to physical characteristics of the stimulus e.g. intensity, frequency, rate of presentation (Naatanen, 1992). They are said to be related to the transmission of sensory information from the peripheral sensory system to the cortex e.g. after auditory stimuli, one can detect "brainstem responses" that have a latency less than 50 msec and these deflections have been

shown to correspond to the activation of various nuclei in the brainstem that are associated with the transmission of auditory information. These components are 'compulsory' in the sense that they will be observed in every individual unless the sensory systems in question are impaired. Endogenous ('Late', 'Slow', or 'Cognitive') components are thought to depend largely on psychological variables and are longer in latency (> 200 msec). They are thought represent activity at the level of the cortex. These components can vary as a function of such factors as attention, task relevance, and the nature of the processing required by the stimulus and some can even be elicited by the absence of an external event, as, for example, when an expected stimulus does not occur.

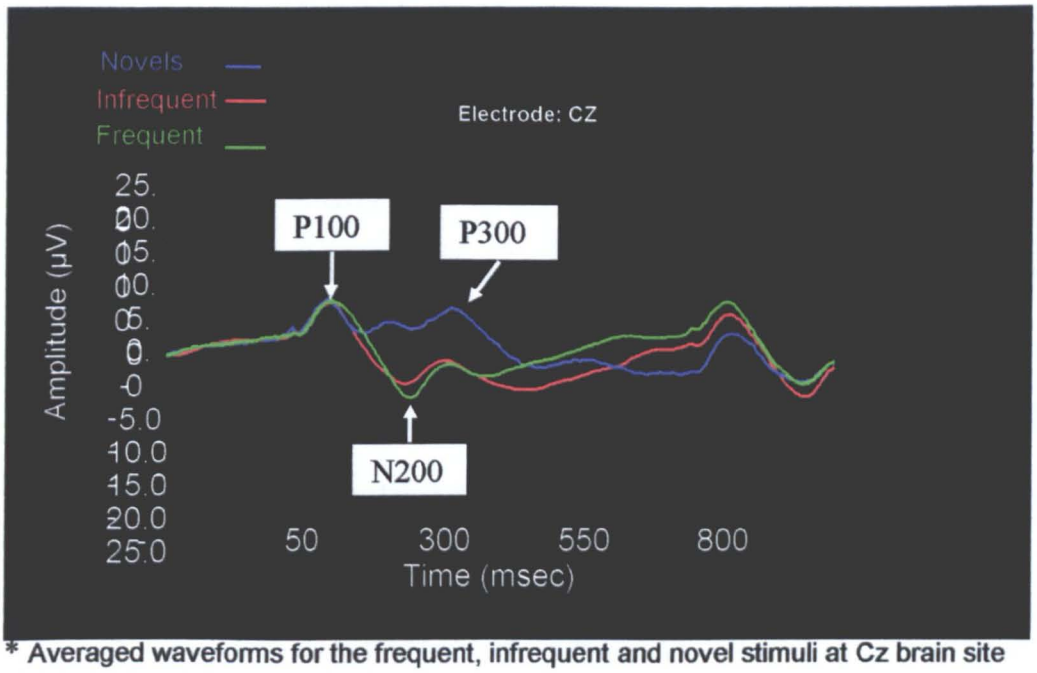
3.3. ERPs as a surrogate marker of cognitive impairment

Event Related Potentials have been used to study aspects of information processing such as selective and sustained attention that are relatively inaccessible using traditional neuropsychological techniques (Pfefferbaum et al., 1995). They have been found to have high test-retest reliability, including in children, supporting their utility in developmental and clinical research (Raikkonen, Birkas, Horvath, Gervai, & Winkler, 2003). The oddball paradigm is frequently used in clinical ERP studies of cognitive deficits and includes the presentation of a series of stimuli with one frequently repeated stimulus, and one infrequent stimulus (Reinvang, 1999). Further, ERPs may be useful because they have been used over various age groups and are sensitive to brain activity (Courchesne, 1978, 1990; Nelson et al., 1998; Ponton et al., 2000). ERPs correlate with neuropsychological tests. For example, in adults with closed head injury, a reduction in the P300 latency correlated with improvement in neuropsychological test scores for short-term and long-term story

recall and for word recall, whilst N200 latency shortening correlated with improvement in the neuropsychological test scores for word recall (Keren, Ben-Dror, Stern, Goldberg, & Groswasser, 1998). In children with behaviour problems, there was a significant correlation between P300 amplitude and Stroop test performance (Kim, Kim, & Kwon, 2001). The group with behaviour problems had smaller P300 amplitudes than the controls. ERPs are not dependant upon language (Byrne et al., 2001; Nelson et al., 1998), can be used in infants (de Haan & Thomas, 2002) and newborns (deRegnier, 2005).

In most ERP 'oddball' studies on adults, subjects are required to actively respond to the target stimulus either by depressing a button or by counting. The target stimulus typically elicits a P300 (also called P3b) waveform that is maximal over the parietal region. However, in a 'passive' paradigm, whereby the intentional discrimination between stimuli is not required, a P300-like waveform (called P3a) is elicited (Ford, Roth, & Kopell, 1976; Jeon & Polich, 2001; Polich, 1986, 1987; Squires, Squires, & Hillyard, 1975). This method is of interest since it can be used in non-compliant or low-functioning children. The 'P3a' or novelty P300 can be obtained from a 3-stimulus paradigm (Figure 3.1) in which distractor (novel) stimuli is inserted into the sequence of target and standard stimuli (Polich, 2003; Squires et al., 1975). These 'novel' stimuli could be a dog bark or car hooting in the auditory paradigm or abstract colour forms in the visual paradigm (Gumenyuk, Korzyukov, Alho, Escera, & Naatanen, 2004; Gumenyuk et al., 2001; Hogan, 2003). Adult studies have showed that the P3a response might be associated with an orienting or investigative response (Escera, Alho, Winkler, & Naatanen, 1998; Friedman & Simpson, 1994).

Figure 3.1: Children's long-latency ERPs to auditory stimulus*

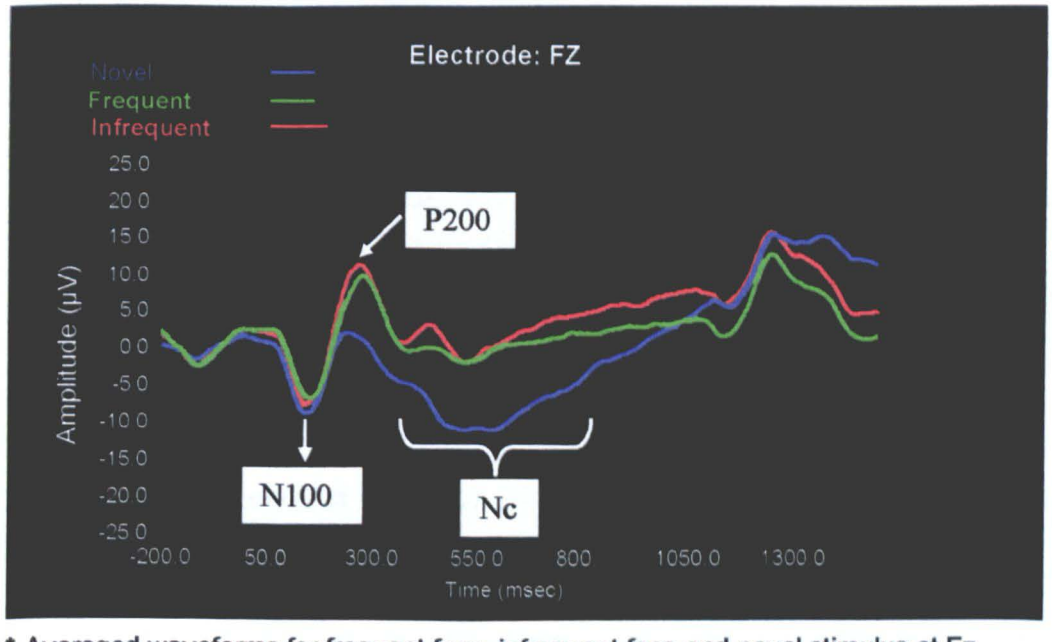


* Averaged waveforms for the frequent, infrequent and novel stimuli at Cz brain site

Children's auditory ERPs are dominated by the P100, N200 and negative component, Nc (Ceponiene, Cheour, & Naatanen, 1998; Courchesne, 1978) (Figure 3.1). The auditory P100 component occurs between 60 and 130 msec after stimulus presentation and is usually interpreted as a neurophysiologic indicator of preferential attention to sensory inputs and is thought to reflect the arousal level (Key, Dove, & Maguire, 2005). The N200 is typically evoked 150 to 250 msec following the presentation of a specific auditory stimulus also dominates auditory ERPs of children. It is said to result from a deviation in form or context of a prevailing stimuli (Naatanen & Picton, 1986). The N200 component in children is thought to be generated by the frontal and parietal cortical fields (Gomot, Giard, Roux, Barthelemy, & Bruneau, 2000) and also in the Heschl's gyrus (Takeshita et al., 2002).

The visual ERPs of children are characterized by the N100, P200, a negative component (Nc) and a slow positive component (Courchesne, 1978; Courchesne, Ganz, & Norcia, 1981; Herbert, Gordon, & McCulloch, 1998; Parker & Nelson, 2005) (Figure 3.2). The visual N100 component occurs between 100 to 200 msec over the frontal-central sites while the P200 is a positive peak between 200 and 300 msec. The N100 and P200 amplitudes are thought to index sensory processing, arousal and attention (Courchesne, 1978). The presence of novel stimuli in a paradigm elicits a large, frontally maximal negativity, called the Nc (Courchesne, 1978; Thomas & Nelson, 1996) with a peak latency between 400 and 800 msec. The Nc occurs over the anterior scalp and is thought to be generated by the frontal lobe and it has been suggested to reflect processing of stimuli that engage one's attention (Courchesne, 1990; Courchesne et al., 1981; Nelson, 1994).

Figure 3.2: Children's long-latency ERPs to visual stimulus*



* Averaged waveforms for frequent face, infrequent face and novel stimulus at Fz

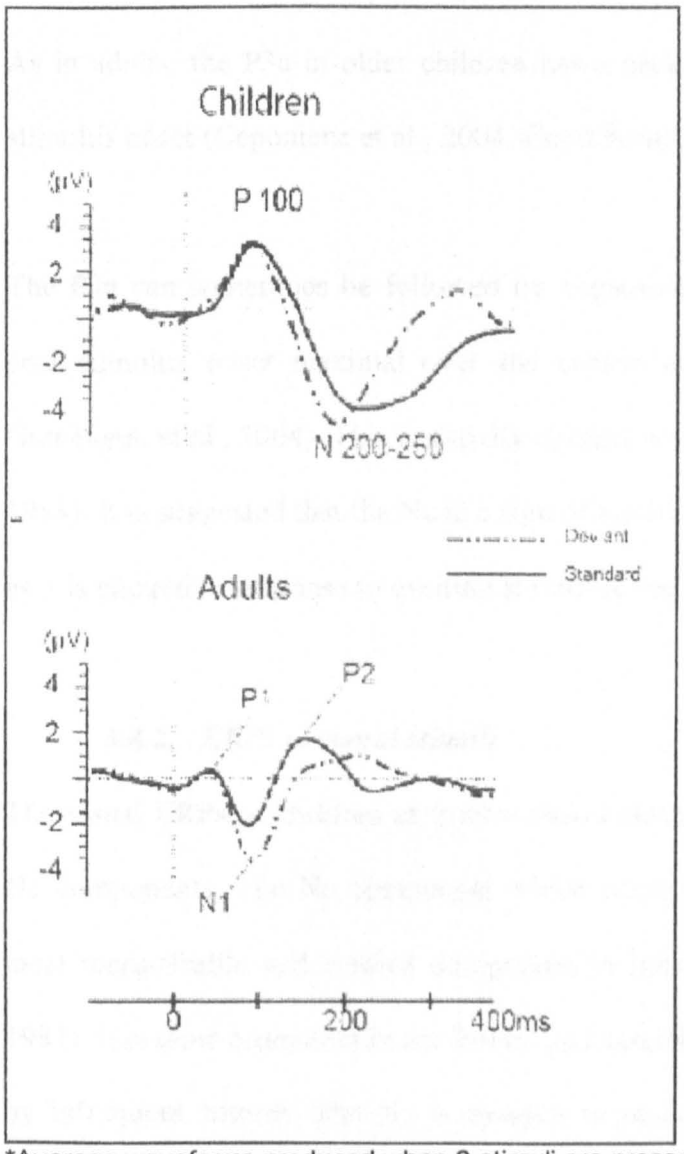
3.4 Developmental changes in cognitive ERPs

3.4.1. *ERPs to auditory stimuli*

In adults, auditory ERPs start with a small P100 (about 50 msec post stimulus onset) followed by a larger N100 response (about 100 msec) and further by a P200 component at approximately 180-200 msec (Naatanen, 1992; Ponton et al., 2000). The P200 peak is followed by a negative component (Ponton et al., 2000) called the N200 at around 220-270 msec. The adult P100-N100-P200 complex, as it is called, is not readily identifiable in infants and children before about 10 years of age (Ceponiene et al., 2001; Kushnerenko, Ceponiene, Fellman, Huotilainen, & Winkler, 2001; Ponton et al., 2000). However, if an inter-stimulus interval longer than one second is used, it is possible to obtain these components in children as young as 3 years old (Ceponiene et al., 1998; Ceponiene et al., 2001; Ceponiene, Yaguchi et al., 2002; Courchesne, 1978).

Children's cognitive ERPs are dominated by the P100 (a positive peak around 100 msec) and N200 (a negative going peak around 200 msec post stimulus presentation) (Ceponiene et al., 1998; Ceponiene et al., 2001; Ceponiene, Yaguchi et al., 2002; Courchesne, 1978). The P100 is the most dominant peak in early childhood (1-4 years) (Kushnerenko et al., 2002) while the N200 becomes more prominent in middle childhood (3-7 years) (Courchesne, 1978) and dominates until adolescence (Ponton et al, 2000). The amplitude of the N200 increases with age from 4-10 years and thereafter decreases. The ERPs of adults, in contrast to those of children are dominated by the P1-N1-P2 complex (Reinvang, 1999) [Figure 3.3].

Figure 3.3: Developmental changes in auditory cognitive ERPs using a 2-stimulus oddball paradigm*



*Average waveforms produced when 2-stimuli are presented at different frequency to children and adults

For the N100 elicitation in children (4-8 years), the auditory stimulus would have to be presented with an inter-stimulus interval of 1sec or longer (Ceponiene et al., 1998). Another component that can be obtained in the passive auditory "oddball" paradigm is the P3a. This component is maximal over the fronto-central cortex and is elicited by stimuli that catch attention (Courchesne, Kilman, Galambos, & Lincoln, 1984; Squires et al., 1975). When 'novel' sounds (e.g. dog bark, colour forms, mechanical noises) are presented as distractors in a series of pure tones, a

P3a component is elicited. The P3a has been proposed to be an electrophysiological marker of the orienting response (Sokolov, Spinks, Naatanen, & Lyytinen, 2002). As in adults, the P3a in older children has a peak latency of 250-350 msec from stimulus onset (Ceponiene et al., 2004; Courchesne, 1990).

The P3a can sometimes be followed by negativity (Nc) at around 500-600 msec post stimulus onset maximal over the central region (Ceponiene et al., 2004; Gumenyuk et al., 2004). This negativity decreases with increasing age (Courchesne, 1983). It is suggested that the Nc is a sign of enhanced auditory and visual attention as it is elicited in response to exciting stimuli (Courchesne, 1978).

3.4.2. *ERPs to visual stimuli*

The visual ERPs of children at fronto-central electrodes are dominated by N1-P2-Nc components. The Nc component which occurs between 400-800 msec is the most recognizable and studied component in infant research (Courchesne et al., 1981). It is most prominent in the frontal and central electrode sites and is produced by infrequent stimuli. The Nc is thought to represent recognition processes (de Haan & Nelson, 1997; Nelson & De Haan, 1996) as it is greater for familiar than unfamiliar faces and for familiar than novel toys. The N100 is the earliest endogenous potential and is linked with processing of spatial information (Rossion et al., 1999). It is maximal in the frontal and central regions and inverts in the occipital to give a P100 component. The amplitude of N100 varies with the amount of attention. The visual P200 component is characterised by a positive shift at the frontal electrodes around 150-250 msec after stimulus onset (Heslenfeld, Kenemans, Kok, & Molenaar, 1997) and a negative component at the occipital

sites. The amplitude of the visual P200 increases with the complexity of the stimulus (Pernet et al., 2003).

3.5. ERPs as a cross-cultural assessment tool

Event related potentials have the advantage of being time-locked to some discretely presented event and thus they provide excellent temporal resolution. In addition, hundreds of trials can be present in a matter of minutes and the participant need not respond verbally or motorically (Byrne et al., 2001; Nelson & Bloom, 1997). This method has proven extremely useful in examining cognitive abilities of special populations such as infants. If ERPs can be shown to be a marker of cognitive function in different cultures, particularly those that do not have standardized neuropsychological tests, then they can be used to identify children at risk of cognitive impairment following cerebral insult.

3.6. Summary

ERPs are on-line processing measures that reflect information-processing stages in a time window extending from sensory processing to complex cognitive events. They are non-invasive in that no chemical or electrical agent is applied to the patients. They have been shown to be useful techniques in assessing cognitive functions in children of different ages and across different cultures. ERPs can also be used in pre-verbal children and those with impaired communication abilities. Their application to children living in areas in which there are no standardized tests has not been assessed, yet they may be particularly influential in this context.

CHAPTER 4

Study Aims, Participants and Methodology

4.1. Main study aims

I set out to establish the use of event related potentials (ERPs) to assess cognitive function in children living in rural Kenya for whom there are few standardized neuropsychological tests. I used the ERPs to assess cognitive impairment in children with a history of exposure to CNS infections including severe falciparum malaria, acute bacterial meningitis (ABM) and HIV infection since they assess information processing independent of language and can be used over a wide range of ages.

The objective of the study is to demonstrate that ERPs can be used to detect neurocognitive impairment following the most common CNS infections affecting children in sub-Saharan Africa, namely falciparum malaria, ABM and HIV.

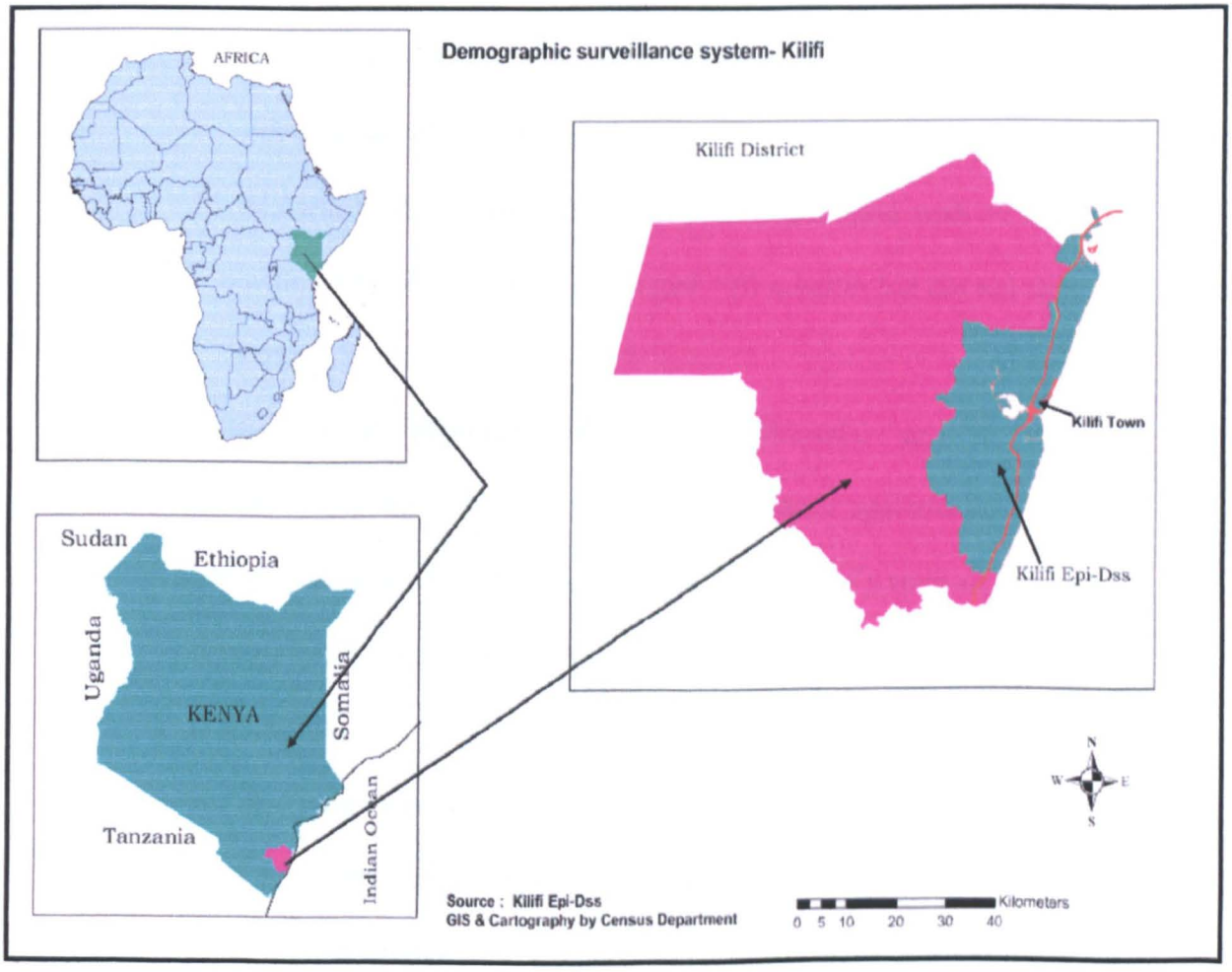
4.2. Specific Objectives

1. To determine the influence of sex, age, socioeconomic status, nutrition and schooling on ERP components to auditory and visual stimuli in children living in rural Kenya
2. To examine cognitive impairment in children exposed to falciparum malaria, pneumococcal meningitis and HIV using ERPs as a surrogate marker.

4.3. Study Site

The study site is located in Kilifi District, on the Kenyan coast, at the Centre of Geographical Medicine Research (Coast)/ Kenya Medical Research Institute (KEMRI). The study subjects live within the Kilifi epidemiological demographic surveillance system (EPI-DSS) (Figure 4.1). The EPI-DSS has a population of about 260,000 people and its records are maintained at the KEMRI centre. A field team three times per year follows up all births and deaths, in-migration and out-migration within the EPI-DSS. The Kilifi District Hospital (KDH) is the main government hospital for this region, and 80% of the admissions to the paediatric ward came from the Kilifi EPI-DSS.

Figure 4.1: Map of Africa, Kenya and Kilifi DSS



4.4. Participants

The children who were admitted to KDH with severe falciparum malaria and ABM, and living in the study area were identified from the admissions to KDH, which have been documented on a standard proforma since 1990. Children exposed to HIV were recruited from the Comprehensive Care and Research Centre (CCRC) at the KDH. The three groups were identified having fulfilled the following inclusion criteria:

A. Severe falciparum malaria defined as children with either cerebral malaria (CM) or malaria with seizures
   a) CM defined as a Blantyre coma score (Molyneux et al., 1989) of ≤ 2 for 4 or more hours, a peripheral parasitaemia and the exclusion of other causes of encephalopathy (Newton et al., 2000).
   b) Malaria with seizures defined as a child admitted with >2 seizures within 24 hours, focal or prolonged >30 minutes but did not develop coma.

B. Pneumococcal meningitis defined as bacterial growth on the cerebrospinal fluid (CSF) culture, positive latex agglutination test for *Streptococcus pneumonia*, organism seen on Gram's stain or white cell count >50 cells/mm^3 or, CSF to blood glucose ratio < 0.1 (Berkley et al., 2001)

C. Human immuno-deficiency virus (HIV) infected children having the virus vertically passed on to them by infected mothers. The HIV status for children above 18 months was tested using UniGold, while

polymerase chain reaction (PCR) was used for those below 18 months. These children have positive antibody tests at 6 weeks, 6 months and 18 months.

Children were excluded from the study if they were found to have gross neurological dysfunction and they were referred to the neuro-clinic at the KDH for treatment.

Age- and sex- matched controls were randomly selected from the EPI-DSS census database of children living in the area. These were screened using the Ten Questions Questionnaire [Appendix 1, (Mung'ala-Odera, Meehan et al., 2004)] to recruit those without neurological impairment. Also they had not been admitted to hospital with a disease with CNS involvement.

Children were excluded if their parents or guardians did not give written informed consent, the children themselves declined participation, or they had acute febrile illness on the day of assessment. All children spoke a Mijikenda language as their first language mainly Kigiryama. Those who were involved in the recording of ERPs were blinded to the group status of these children until after the analysis of the data.

4.5. Ethical issues and informed consent

Permission to carry out the study was obtained from the National Ethical Review Committee (ERC) of Kenya. Informed consent was obtained from the child's parent or legal guardian before the start of the assessment procedure. A field worker who

explained the study to the parent/guardian visited the homes of the control children who were randomly selected. If informed consent was given, the parent was asked to bring the child to the KEMRI assessment centre where all the tests were performed.

4.6. Procedures

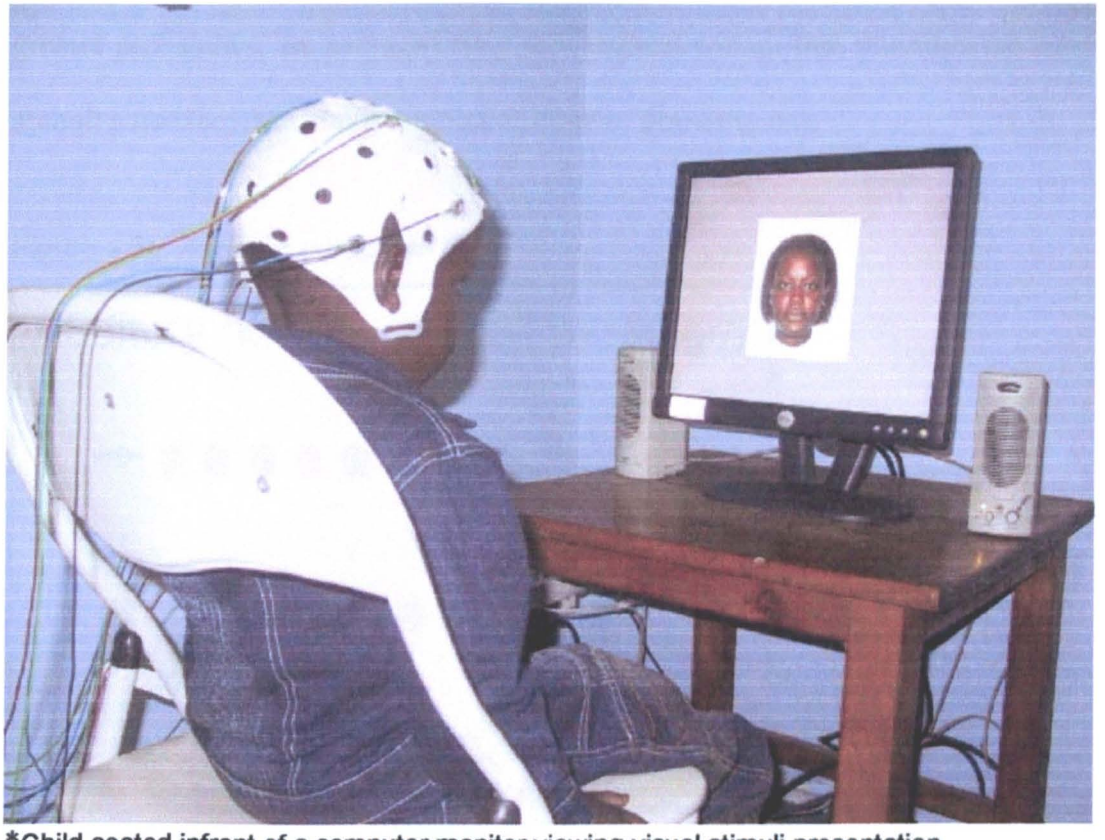
Since successful assessment depended on the children's ability to see and hear properly, each child had hearing tested with a Kamplex screening audiometer (PC Werth, UK) and vision tested by Sonksen-Silver chart (Salt, Sonken, Wade, & Jayatunga, 1995) or Keller Cards to detect any visual and auditory impairments. Those with visual or hearing impairments were excluded from the particular paradigms they had impairments in. Each child had auditory and visual ERPs recorded.

4.7. ERP Test Battery

4.7.1 Novelty Processing

The stimuli consisted of auditory and visual paradigms that were presented using *Presentation* software (Neurobehavioral Systems). Continuous EEG data was recorded using Neuroscan® version 4.3 (Compumedics Limited®, USA). Each child sat on an easy chair in a partially lit, sound-attenuated room looking towards a computer monitor placed 70 cm away (Figure 4.2). Both auditory and visual paradigms were passive novelty oddball protocols, consisting of frequent, infrequent and novel stimuli.

Figure 4.2: Recording visual ERPs in the laboratory*

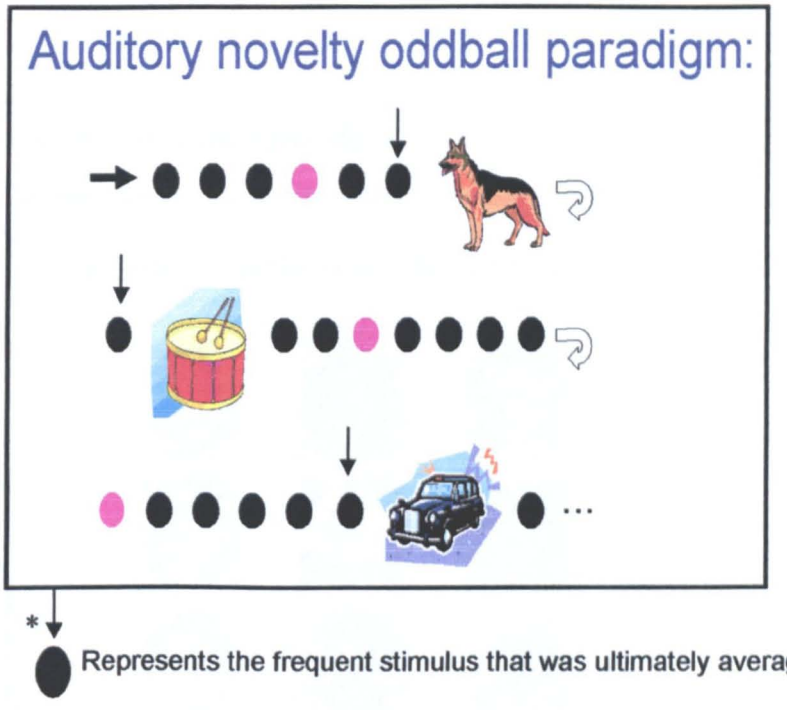


*Child seated infront of a computer monitor viewing visual stimuli presentation

The passive auditory paradigm was composed of frequent tones (figuratively represented by a black dot), infrequent tones (pink dot) and novel sounds (environmental noises) [Figure 4.2a]. This paradigm was developed by Torsten Baldeweg (Institute of Child Health, London) and previously used by Alex Hogan (Hogan, 2003). These tones/sounds were presented through two speakers placed in front of the children next to the monitor using *Presentation* software. Twelve percent of the stimuli were infrequent tones of 2000 Hz (70dB sound pressure level, SPL), 12% were composed of novel noises e.g. dog bark, bell ring etc whereas the remainder were frequent tones of 1000 Hz (70dB SPL). The duration of the tones/noises was 100 milliseconds (msec) with an onset to onset (stimulus onset asynchrony) of 700 msec. Subjects were not given any instructions other than to listen to the sounds. The total time taken for the auditory task was 8 minutes with

an average of 650 stimulus presentations. Since the frequent stimulus had a much greater probability, we averaged only the frequent stimuli that preceded the novel stimuli since they had almost similar number of presentations.

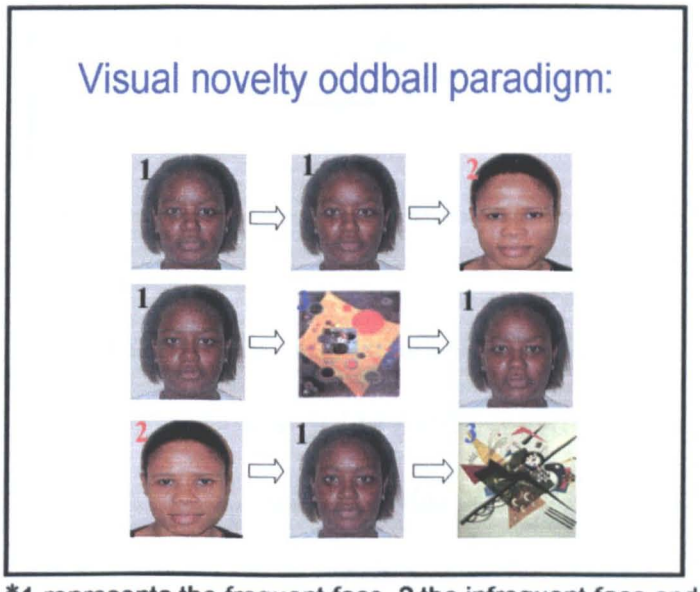
Figure 4.3a: *Auditory paradigm**



The visual paradigm consisted of 2 female faces (a frequent and infrequent occurring face) posing with neutral expressions. Subjects also saw trial unique, non-face novel stimuli of abstract patterns (Kandinsky's paintings) [Figure 4.3b]. This paradigm was adapted from Alex Hogan (Hogan, 2003) but the faces in her paradigm were substituted with those of local African women. A pilot study of 10 school-going local children showed that the use of trial-unique faces as novels did not produce a large frontal negative component as expected. The paradigm was modified to include trial-unique abstract paintings in place of the trial-unique faces. The inclusion of the abstract paintings produced a large frontal negativity (see Figure 3.2, pg 70). All stimuli were in colour photographic images presented on a

computer screen and were of equal size and visual angle (13.0 cm by 15.0 cm, viewed from a distance of 70 cm). Two blocks of 100 trials were presented in a random order, with 60% of the trials showing the frequent face, 20% of the infrequent face, and 20% novel stimuli. Subjects were not given any instructions other than to watch the computer screen and minimize movement.

Figure 4.3b: *Visual paradigm**

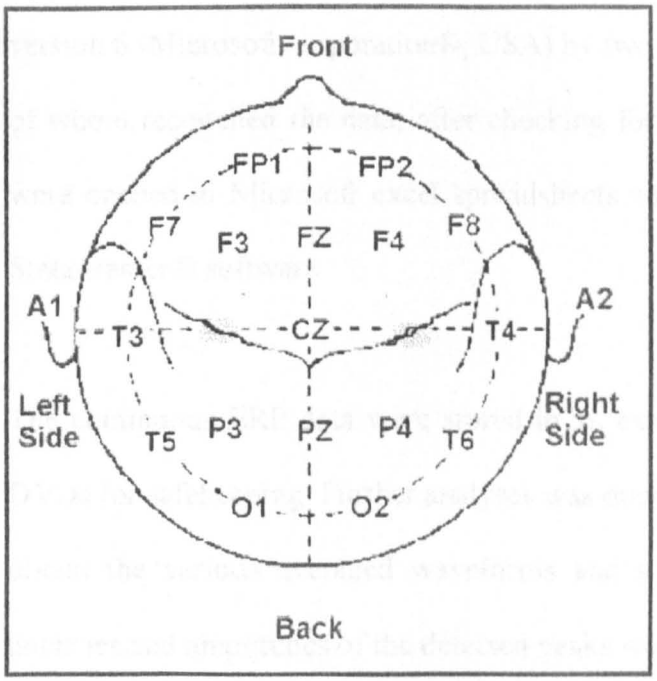


*1 represents the frequent face, 2 the infrequent face and 3 the novel stimuli

4.7.2 ERP recordings

The ERPs were recorded from 18 scalp electrodes positioned using standard 10-20 system. Data were recorded from midline leads at Fz, Fcz, Cz and Pz, as well as lateral leads at Fpl, Fp2, F7, F8, T3, T4, T5, T6, P3, P4, O1, O2 and mastoid processes (A1, A2) (Figure 4.3). Horizontal and vertical electro-oculographs (EOG) were monitored by two electrodes on the outer canthus of the eye and just below the eye respectively.

Figure 4.4: Electrode placement schedule



All locations were referenced to a common Cz reference and later re-referenced to averaged mastoids [(A1+A2)/2] reference for purposes of comparison with previous studies employing this reference. All auditory data were collected using a 1000 msec recording epoch with a 200 msec pre-stimulus baseline, while the visual data had a 1500 msec recording epoch and a 200 msec baseline. All impedances were less or equal to 8.2 kΩ. EEG and EOG channels were recorded using Neuroscan® version 4.3 acquisition system and NuAmps amplifier (band-pass 0.1 to 70 Hz). Continuous EEG data were recorded at a sampling rate of 500 Hz and low pass filtered off-line at 20 Hz. Epochs were corrected for baseline and excluded if they exceeded 100 μV in either direction.

4.7.3 Data management and analysis

Background information, clinical histories and biodata of the children were obtained and entered using specifically designed proformas (See Appendix 4-7).

These data were then entered independently into a database using Visual FoxPro® version 6 (Microsoft corporation®, USA) by two independent data entry clerks, one of whom reconciled the data, after checking for any discrepancies. The data files were opened in Microsoft excel spreadsheets and then transferred to SPSS using Stata-tranfer® software.

The continuous ERP data were stored in an external hard disk and backed up in DVDs for safekeeping. Further analyses was done using Neuroscan® version 4.3 to obtain the various averaged waveforms and to detect the required peaks. The latencies and amplitudes of the detected peaks were stored in an Excel (Microsoft®, USA) spreadsheet.

The latencies and amplitudes for these components were automatically detected from baseline using the "peak-detection" feature of the *Neuroscan* software and exported to a spreadsheet for analysis. A manual check of the peaks automatically detected was carried out and those not placed on peaks were manually corrected. Data analysis was carried out using SPSS for Windows version 13 (SPSS Inc®, Chicago, USA). Mixed ANOVAs was used to determine main and interaction effects of the various independent variables (e.g. Age, Sex, Schooling, nutrition, occipito-frontal head circumference and Socioeconomic status) and dependent variable (Diagnosis) on the outcome measure (cognitive performance). Within-subject factors included electrode (X4 – Fz, Fcz, Cz and Pz), stimuli (X3 – frequent, infrequent and novel). The between-subject factors were Age, Sex (male, female), Socioeconomic status and Schooling

Socioeconomic status was determined by mother's educational status. Maternal education is the best single predictor of child health in resource poor countries (Bollen, Glanville, & Stecklov, 2001; Bradley & Corwyn, 2002) and co-varies with socioeconomic status (Wachs & McCabe, 1998). It has been found to be a good predictor of socioeconomic status in the area (Penny Holding, personal communication). The occipito-frontal head circumference (OFC) was measured using a tape measure by the ERP technician.

The nutrition status of the children was determined using anthropometric indices of weight and height in reference to age and sex (de Onis, Blossner, Borghi, Morris, & Frongillo, 2004). Classification of z-scores for stunting (height for age), wasting (weight for height) and underweight (weight for age) were computed using Epi-info® version 3.4.1 (WHO, 1978 reference).

The Greenhouse-Geisser correction was used to correct for sphericity where applicable. We used the Tukey-Kramer test to correct for unequal sample sizes in the *Post-hoc* analyses. Level of significance was set at $p < 0.05$.

CHAPTER 5

Investigation of the long latency event-related potentials in normal Kenyan children

5.1. Introduction

Children living in sub-Saharan African are exposed to multiple risks including poverty, malnutrition and poor health, all of which have detrimental effect on their cognitive development (Grantham-McGregor et al., 2007). There is generally a lack of monitoring and evaluation of these effects due to lack of appropriate neuropsychological tools for assessment (Holding et al., 2004). The development of assessment tests that are suitable and culturally fair for the local population need to accommodate; a lack of familiarity with test demands due to high illiteracy rates, language barriers, poor interpersonal skills (since children rarely interact with strangers) and a shortage of trained psychologists to administer the tests.

The aim of this study is to examine the development of both the visual and auditory ERP response to novelty in children aged 4-12 years of age living in rural Kenya and to determine which factors influence these responses. The study was designed to determine the suitability of ERPs for assessing cognitive function in a rural setting. The ERP component is elicited using the oddball paradigm, wherein two stimuli of differing frequencies are presented in random order. The subject is required to discriminate an infrequent stimulus from the frequent stimulus either overtly or covertly (Polich, 2004). The 'novelty oddball' ERP task is a modification of the typical oddball task whereby three types of events are presented: (a) a

stimulus that is repeated at high probability (also referred to as the 'frequent stimuli'), (b) a stimulus that is repeated at low probability (referred to as the 'infrequent stimuli') and (c) a set of trial-unique novel stimuli presented at low probability. A distinct waveform for the novel events is observed for both visual and auditory events and can be obtained even in passive tasks where the participant need only look at or listen to the stimuli without performing an active response (Courchesne, 1978; Picton, 1992; Polich, 1986, 1987; Polich & McIsaac, 1994; Squires et al., 1975). These characteristics of the ERP response to novelty make it useful for studying cognitive development in young children and in those where language constraints make use of more traditional neuropsychological assessments difficult.

Although the auditory response to novelty stimuli in children have been reported, relatively few studies have systematically examined how the response changes with age or included children younger than 6 years old, and even fewer have examined response to novelty in both visual and auditory modalities in the same study. Given that many investigators are interested in how atypical responses to novelty can inform our understanding of developmental disorders including autistic spectrum disorders (Ferri et al., 2003; Oades, 1998), attention deficit disorder (Kilpelainen et al., 1999; Sangal & Sangal, 2004), further understanding of how the response normally develops is clearly needed. This chapter sets out to present the normative data on the basic ERP components elicited in the passive auditory and visual oddball tasks since no data are available on these components in normal Kenyan children.

5.2. Study area and sampling

One hundred and eighty-seven children aged 4-12 years old were selected from the study area from the community database. None of the children had a history of significant disease, or suffered from cognitive deficits or was taking medication at the time of the study. In total 88 boys (mean= 6 years 11 months) and 99 girls (mean= 6 years 10 months) were randomly selected from the community.

Auditory assessment using Kamplex screening audiometer (PC Werth, London) and visual assessment using a Sonksen-Silver chart (Salt et al., 1995) were administered to each child to ensure their vision and hearing was normal. Refreshments were served to each child before the assessment began. Inclusion criteria were children without a history of severe malaria, HIV or meningitis (with no history of unconsciousness or convulsions). Children had to have normal hearing and vision and the parents/guardians consented to the study.

5.3. Data analysis

Analysis targeted the P100, N200 and P3a components in the auditory "oddball" paradigm. The P100 component was defined as the highest peak between 60 and 130 msec post stimulus presentation while the N200 component was defined as the most negative point between 120 and 220 msec. The P3a component was defined as the most positive point occurring between 250 and 450 msec. In the visual paradigm, we analysed the N100, P200 and Nc. The most negative point between 100-200 msec was defined as the N100, while P200 (P250) was the most positive point between 200-300 msec. The negative component, Nc, was defined as the average amplitude between 300 and 850 msec.

All components were analysed by repeated measures ANOVA using SPSS for Windows, version 13. Within-subject factors included site (X4 – Fz, Fcz, Cz and Pz), stimuli (X3 – frequent, infrequent and novel). The between-subject factors were Age (4, 5, 6, 7, 8, 9, 10-12 years), Socioeconomic status (high or low), Sex (male, female), Nutrition status (normal, malnourished), Head circumference and Schooling (yes or no). Each of the between-subject factors was entered separately into the model to determine its influence on the ERP components. A cut-off value of $p< 0.25$ was used. A more detailed description of methodology is given in Chapter 4.

5.4. Results

Children between ages 10-12 years old were grouped for analysis. Eleven children were excluded since they were found to be over the age range and those of another 6 children in the auditory paradigm were excluded as was those of 4 children in the visual experiment due to excessive artefact. Sixty-eight percent of the children were attending school (at least nursery school). Children whose mothers attended school were recorded as having relatively higher socioeconomic status while those with less than 2 SD on the height for age z-score were recorded as malnourished. A description of the study sample is shown in table 5.1 below:

Table 5.1: Descriptive statistics of the study sample

Age (years)	4	5	6	7	8	9	10-12
N*	21	19	43	34	30	15	8
Male (%)	11 (52%)	8 (42%)	22 (51%)	16 (47%)	9 (30%)	10 (67%)	7 (88%)
Attending school (%)	9 (42%)	9 (47%)	27 (63%)	26 (76%)	20 (67%)	14 (93%)	6 (75%)
Malnourished (%)	35.5%	17.5%	0	9.0%	13.5%	6.0%	14.0%
Relatively high SES (%)	44%	29%	55%	48%	30%	36%	38%

*N represents the number of children in each age group

The univariate analysis of between-subjects factors revealed that Age had significant associations with most of the ERPs components of the children (Table 5.2). Sex was significantly associated with the N200 latency and marginally with the Nc component. The children's SES was significantly associated with the Nc component and marginally with the P3a latency, while head circumference was associated with the latency of the P100 component only. Schooling was not associated with any ERP component in our sample. The results suggested that age should be taken into account in all subsequent ERP analysis and added sex since it had a marginal influence on the components. The other independent variables; SES, head circumference, schooling and nutrition were excluded from analysis of the ERP components while Age and Sex were retained in the ANOVA model.

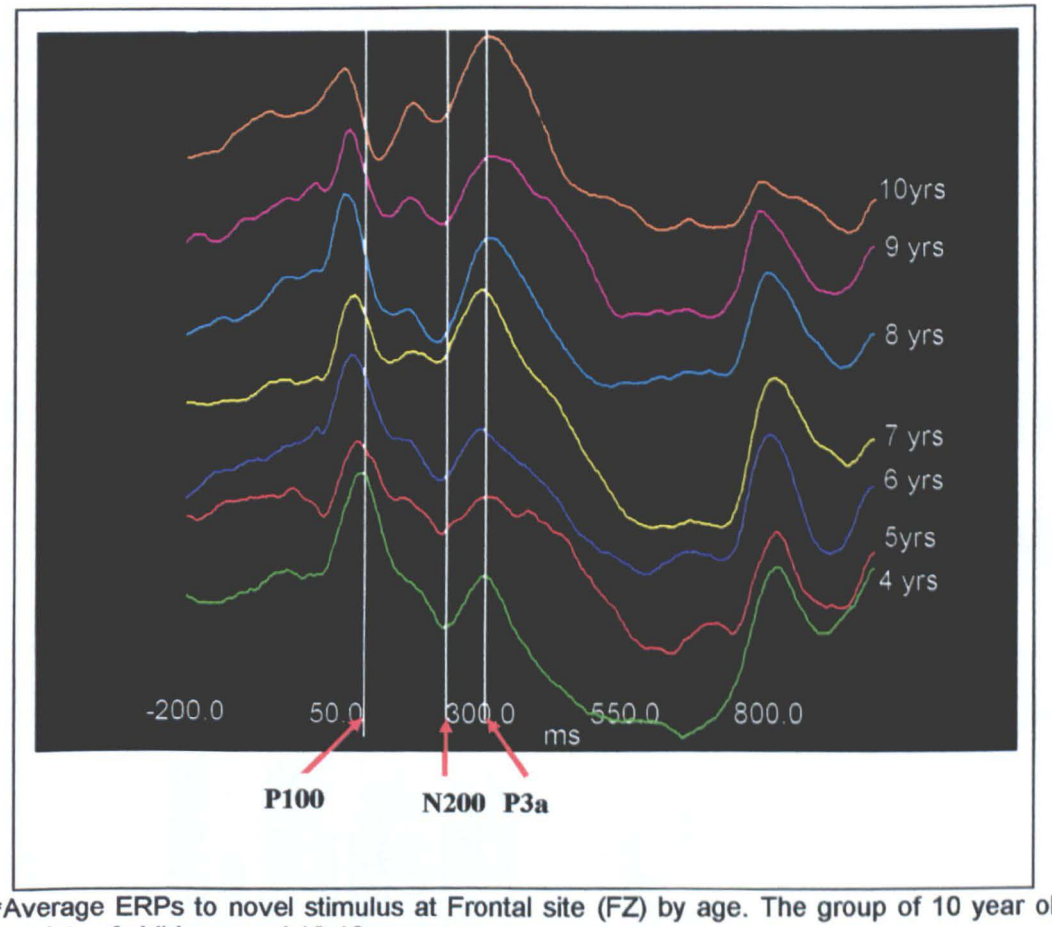
Table 5.2: Effects of independent variables on ERP components

	Variables	df	F	P-value		F	P-value
Auditory							
P100 latency	**Age (4 to 12)**	6	3.418	**0.004**	P100 amplitude	4.391	**0.001**
	Sex (M or F)	1	0.273	0.602		0.018	0.894
	Head Circumference	12	2.164	**0.017**		0.89	0.558
	Schooling (Y or N)	1	0.003	0.954		0.023	0.879
	Mother Educ (SES)	1	0.322	0.572		0.001	0.992
N200 latency	**Age (4 to 12)**	6	3.33	**0.004**	N200 amplitude	1.091	0.371
	Sex (M or F)	1	4.267	**0.041**		1.326	0.252
	Head Circumference	12	1.467	0.145		0.428	0.95
	Schooling (Y or N)	1	0.396	0.53		0.236	0.628
	Mother Educ (SES)	1	0.031	0.861		0.105	0.746
P3a latency	**Age (4 to 12)**	6	3.191	**0.006**	P3a amplitude	0.775	0.591
	Sex (M or F)	1	1.311	0.254		0.074	0.878
	Head Circumference	12	0.676	0.772		0.733	0.717
	Schooling (Y or N)	1	3.042	0.083		0.572	0.451
	Mother Educ (SES)	1	3.187	0.077		1.776	0.185
Visual							
N100 latency	**Age (4 to 12)**	6	3.644	**0.002**	N100 amplitude	4.085	**0.001**
	Sex (M or F)	1	0.636	0.427		0.395	0.531
	Head Circumference	12	0.849	0.6		1.541	0.118
	Schooling (Y or N)	1	0.193	0.661		0.204	0.652
	Mother Educ (SES)	1	0.066	0.798		0.632	0.428
P200 latency	**Age (4 to 12)**	6	1.793	0.105	P200 amplitude	3.504	**0.003**
	Sex (M or F)	1	0.794	0.375		0.123	0.726
	Head Circumference	12	1.458	0.149		0.607	0.833
	Schooling (Y or N)	1	0.686	0.409		1.095	0.297
	Mother Educ (SES)	1	0.012	0.912		1.194	0.277
Nc mean Amp	**Age (4 to 12)**	6	3.082	**0.007**			
	Sex (M or F)	1	2.927	0.09			
	Head Circumference	12	0.566	0.866			
	Schooling (Y or N)	1	0.011	0.915			
	Mother Educ (SES)	1	8.046	**0.005**			

5.4.1 Auditory Paradigm

Analysis focused on the P100, N200, and P3a illustrated for the novel stimulus in Figure 5.1 below. The components underwent changes with age mostly reduction in latencies and increase in amplitudes.

Figure 5.1: Auditory ERPs to novel stimulus by age at Fz*



*Average ERPs to novel stimulus at Frontal site (FZ) by age. The group of 10 year olds consists of children aged 10-12 years

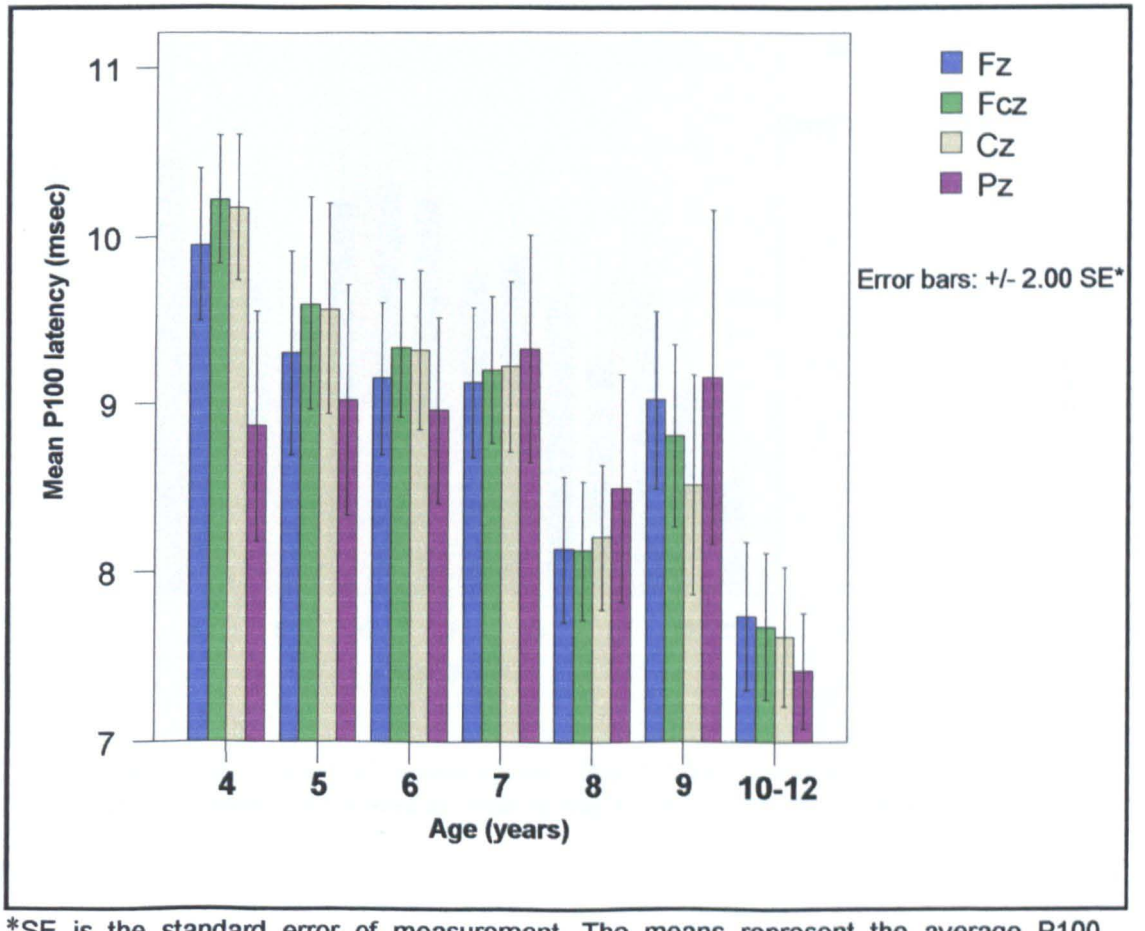
5.4.1.1 Auditory P100

Latency:

There were significant main effects of Stimulus [F (2, 312) = 8.24, p< 0.001] and Age [F (6, 156) = 2.627, p= 0.019] and significant interactions of Stimulus by Age [F (12, 312) = 2.978 (p= 0.001)] and Electrode by Age [F (18, 468) = 3.004 (p= 0.002)] on the latency of the P100. *Post hoc* analysis showed that the main effect of

Stimulus occurred because P100 latency was faster to rare/novel stimuli than the frequently presented stimuli. The main effect of age occurred due to decreasing P100 latencies with increasing age (Figure 5.2). In order to determine the interaction of Stimulus by Age, separate ANOVAs with Stimulus as factor for the different age groups was carried out. The interaction was driven by a decrease in the latency to novel stimulus with increasing age but the same was not true for the frequent and infrequent stimulus. Separate ANOVAs with Electrode as factor for different age groups were used to determine the Electrode by Age interaction. The interaction occurred because the P100 latency decreased by age especially in the fronto-central electrodes.

Figure 5.2: Effect of Age on auditory* P100 latency

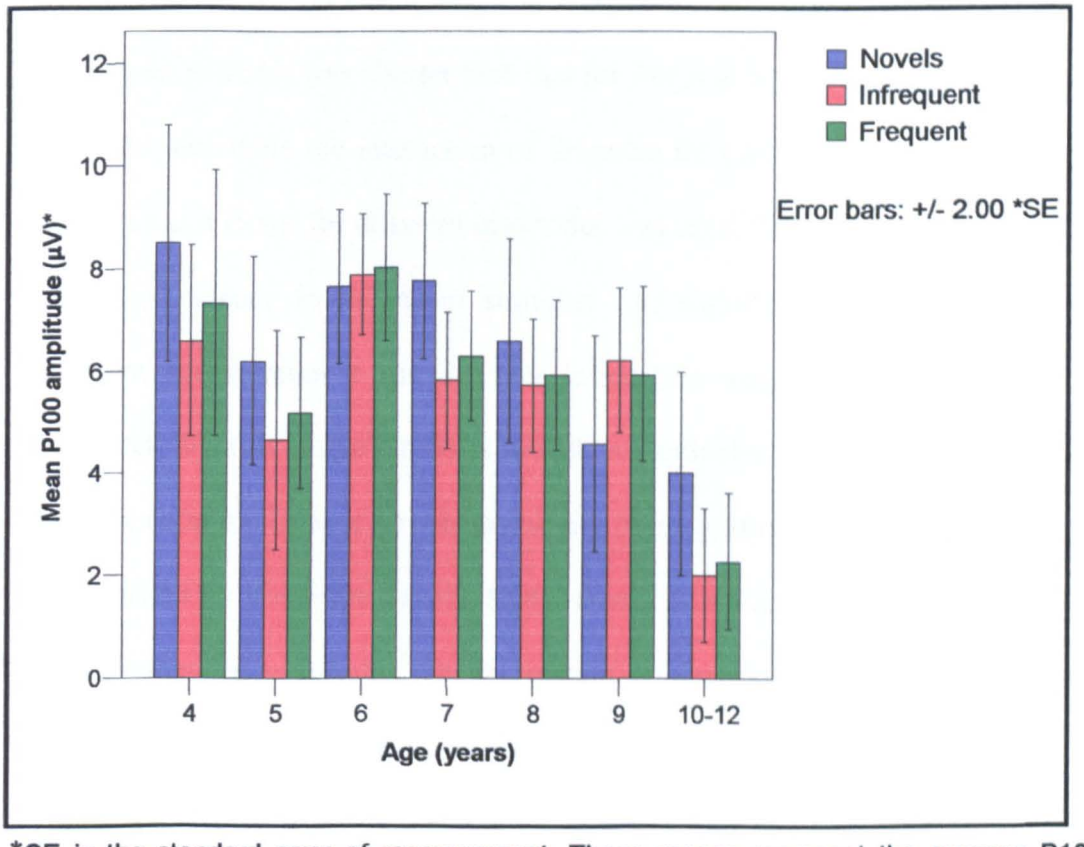


*SE is the standard error of measurement. The means represent the average P100 latencies of community controls to novel auditory stimuli at the mid-line electrodes

Amplitude:

There were main effects of Stimulus [F (2, 312) = 3.579, p= 0.029], Electrode [F (3, 468) = 144.77, p< 0.001] and Age [F (6, 156) = 2.713, p= 0.016]. *Post hoc* analysis showed there was a trend towards larger P100 amplitudes to the novel stimuli but this did not reach significance. The main effect of Electrode occurred because the P100 amplitude had a fronto-central maximum and decreased posteriorly. The main effect of Age was due to a decrease in the P100 amplitude by age between 7-10 years (Figure 5.3).

Figure 5.3: Effect of Age on P100 amplitude



*SE is the standard error of measurement. These means represent the average P100 amplitudes of community controls to novel stimuli at the mid-line electrodes

5.4.1.2 Auditory N200

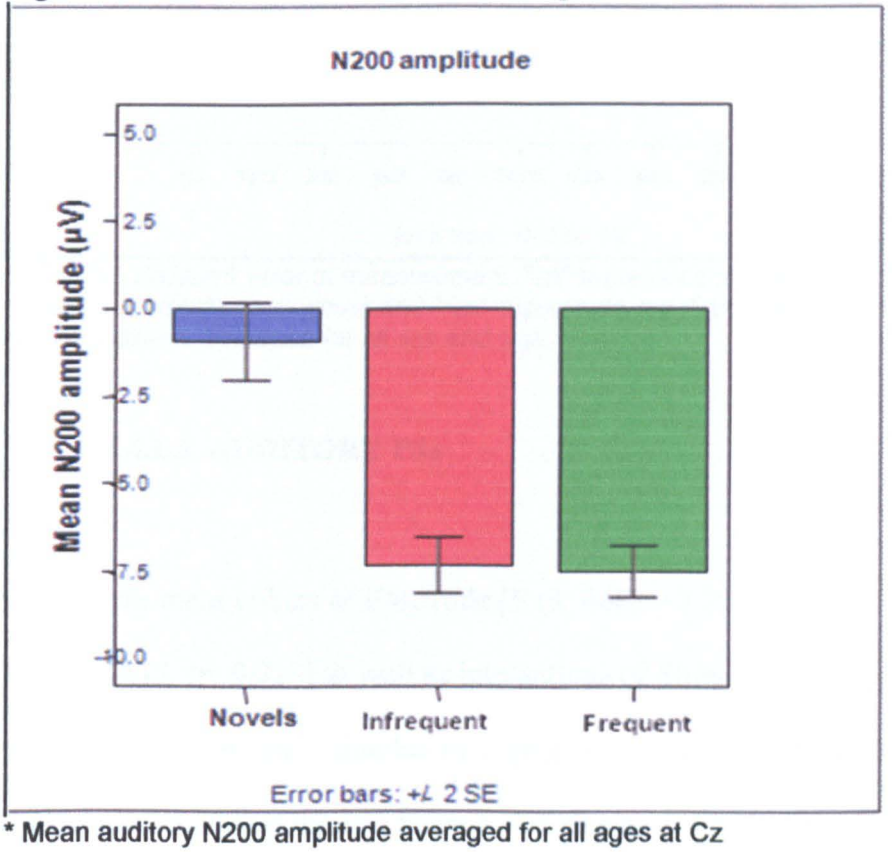
Latency:

There were main effects of Stimulus [F (2, 312) = 4.346, p= 0.017], Age [F (6, 156) = 4.593, p< 0.001)] and Sex [F (1, 156) = 8.967, p= 0.003] on the latency of the N200 and significant interactions of Stimulus by Age [F (12, 312) = 2.343, p= 0.009], Stimulus by Electrode [F (6, 936) = 3.293, p= 0.011], Stimulus by Electrode by Age F [(36, 936) = 1.896, p= 0.006] and Stimulus by Electrode by Sex [F (6, 936) = 3.344, p= 0.010]. *Post hoc* analysis did not reveal a significant effect between different Stimuli on N200. The main effect of Sex occurred because males had shorter latencies than females while that of Age was due to a gradual decrease in N200 latency from 5-10 years. The interaction of Stimulus by Age occurred because the N200 latency to the infrequent stimulus was shorter than that for frequent or novel stimuli for 7- and 8-year olds. To determine the interaction of Stimulus by Electrode, separate ANOVAs with Stimulus as a factor for different electrodes was used. The interaction occurred because the N200 latency to the novel stimulus was significantly longer than that of the frequent and infrequent stimuli. This interaction was further modified by sex and separately by age. In the ANOVA model, we split the file by sex and later by age, to determine their influence on the interaction. Sex significantly influenced the interaction of Stimulus by Electrode due to shorter latencies at Fz, Fcz and Cz electrodes among males compared to females. The interaction of electrode by stimulus was modified by age for children aged 4-8 years with a decrease in the N200 latency to the novel stimulus at Cz.

Amplitude:

The N200 amplitude revealed both a significant main effect of Stimulus [F (2, 312) = 55.746, p< 0.001] and Electrode [F (3, 468) = 22.388, p< 0.001]. An interaction between these two factors (Stimulus by Electrode) was also significant [F (6, 936) = 47.503, p < 0.001]. *Post hoc* analyses showed that the amplitude of the N200 to the novels stimulus was significantly smaller than other stimuli (Figure 5.4).

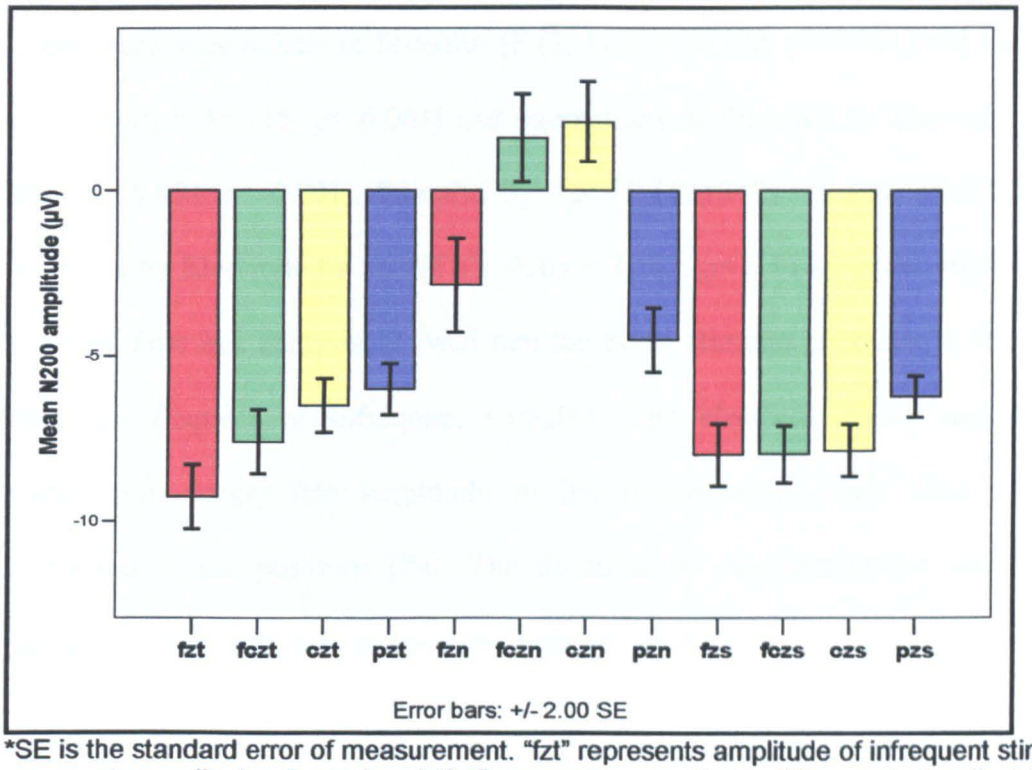
Figure 5.4: Effect of Stimulus on P200 amplitude*



* Mean auditory N200 amplitude averaged for all ages at Cz

The amplitude of the N200 decreased from anterior to posterior electrodes. The interaction effect of Stimulus by Electrode was due to significantly smaller N200 amplitudes to novel stimulus than the frequent or infrequent stimuli over the anterior and central electrodes (Figure 5.5).

Figure 5.5: Interaction effects of Stimulus and Electrode on N200 amplitude



*SE is the standard error of measurement. "fzt" represents amplitude of infrequent stimuli, "fzn" represents amplitude of novel and "fzs" represents amplitude of frequent stimuli at Fz. Mean N200 amplitude averaged for all sex and age.

5.4.1.3 AUDITORY P3a

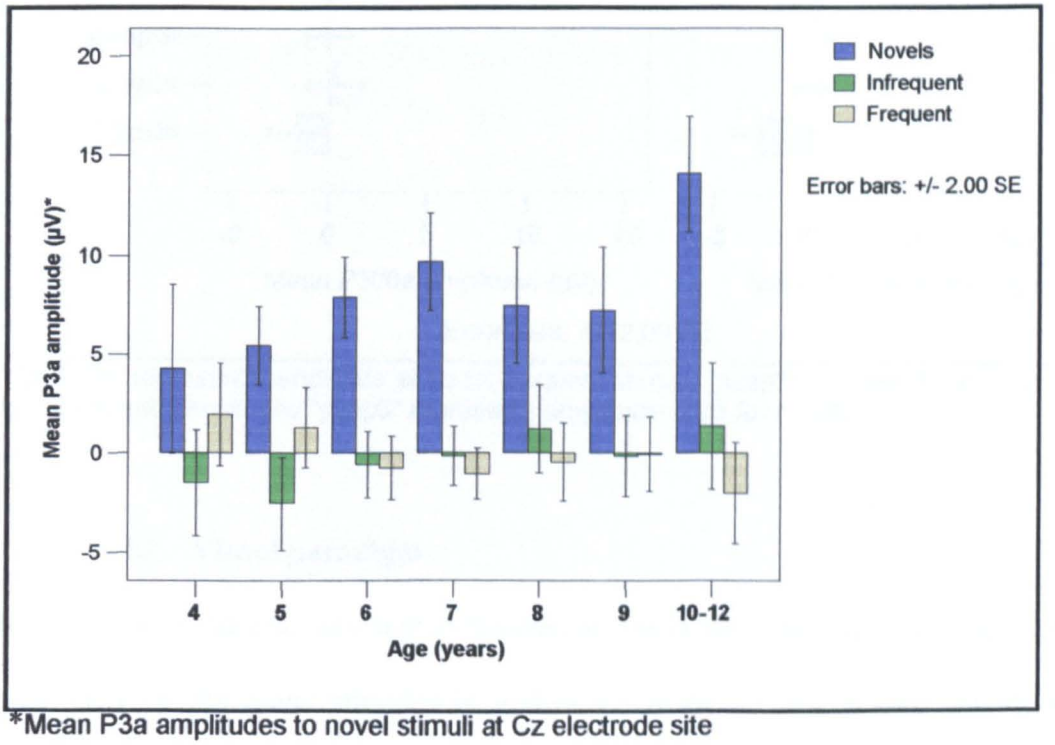
Latency:

There were main effects of Electrode [F (3, 468) = 3.394, p= 0.034] and Age [F (6, 156) = 2.813, p= 0.013] as well as interactions of Stimulus by Electrode [F (6, 936) = 3.751, p= 0.004] and Stimulus by Age [F (12, 312) = 1.848, p= 0.040]. *Post hoc* analysis revealed that the P3a latency was shorter at Cz than Pz. The main effect of Age and the interaction of Stimulus by Age did not significantly influence P3a latency on *post hoc* analysis. The interaction of Stimulus by Electrode occurred because the P3a peak amplitude of the novels was quicker at Cz than at the posterior electrodes while there was no difference in latency across electrodes for other stimuli.

Amplitude:

There were main effects of Stimulus [F (2, 312) = 66.178, p< 0.001] and Electrode [F (3, 468) = 15.118, p< 0.001] and interactions of Stimulus by Electrode [F (6, 936) = 15.954, p< 0.001], Stimulus by Age [F (12, 312) = 2.409, p= 0.007] and Stimulus by Electrode by Sex [F (6, 936) = 3.252, p= 0.018] on the amplitude of the P3a. *Post hoc* analyses showed that the novel stimulus produced a larger P3a than the frequent or infrequent stimulus. The electrode effect was due to significantly larger P3a amplitude in the fronto-central leads (Fcz and Cz) compared to the posterior (Pz). The Stimulus by Age interaction was due to increases in the amplitude to the novel stimulus with increasing age (Figure 5.6).

Figure 5.6: Mean P3a amplitudes* by age

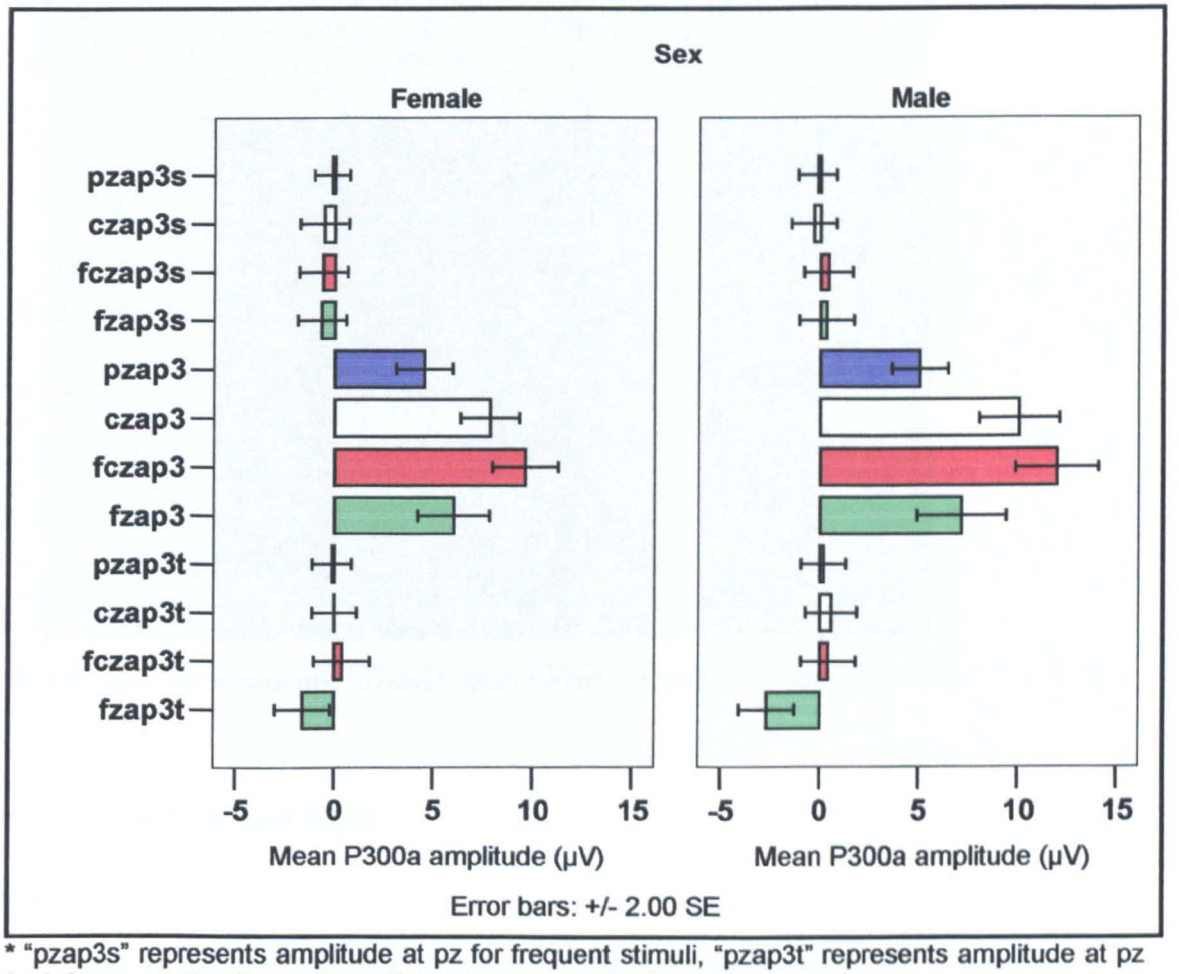


*Mean P3a amplitudes to novel stimuli at Cz electrode site

The Stimulus by Electrode interaction occurred because the P3a amplitude to the novel stimuli was greater at Fcz and Cz than at Pz. This interaction was further

modified by sex with boys having larger P3a amplitudes than girls to the novel stimulus at Fcz and Cz electrodes (Figure 5.7).

Figure 5.7: Interaction of stimulus and electrode and sex on P3a amplitude*

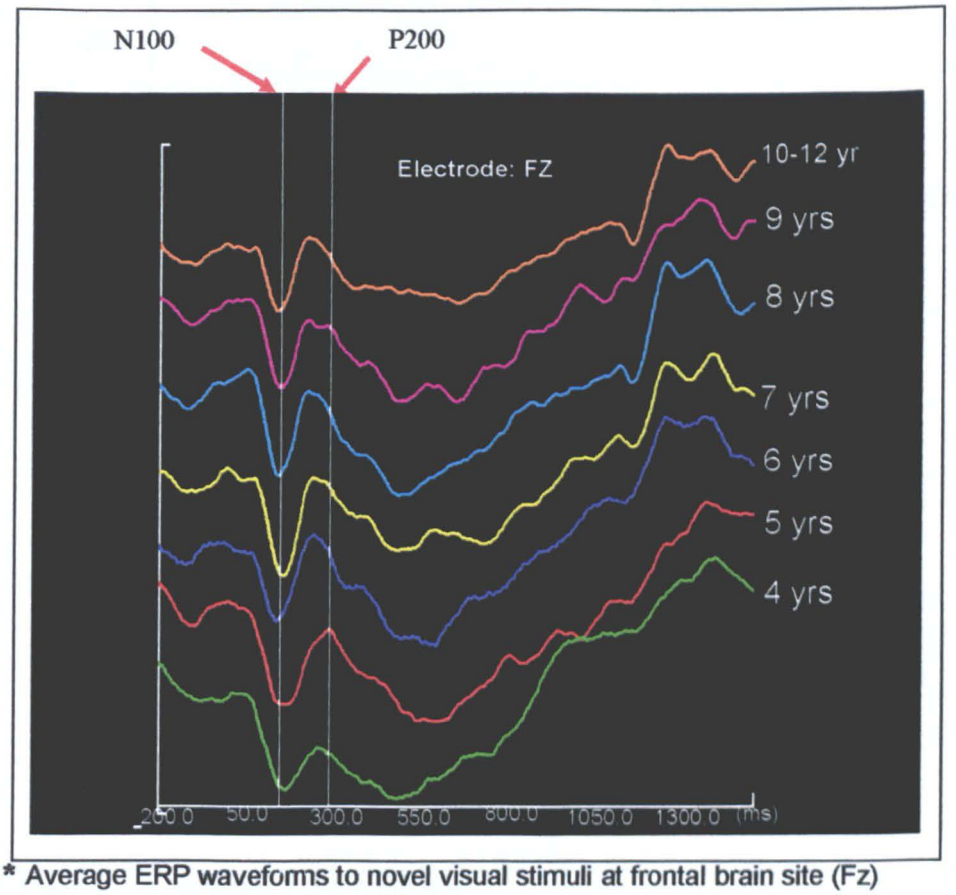


* "pzap3s" represents amplitude at pz for frequent stimuli, "pzap3t" represents amplitude at pz for infrequent stimuli, and "pzap3" represents amplitude at pz for novels

5.4.2 Visual paradigm

In the visual paradigm, the analysis focused on the N100, P200 and Nc components illustrated for the novel stimulus in Figure 5.8 below. The averaged waveforms show a decrease in the P200 latency with increasing age.

Figure 5.8: Visual ERPs to novel stimulus by age at Fz*



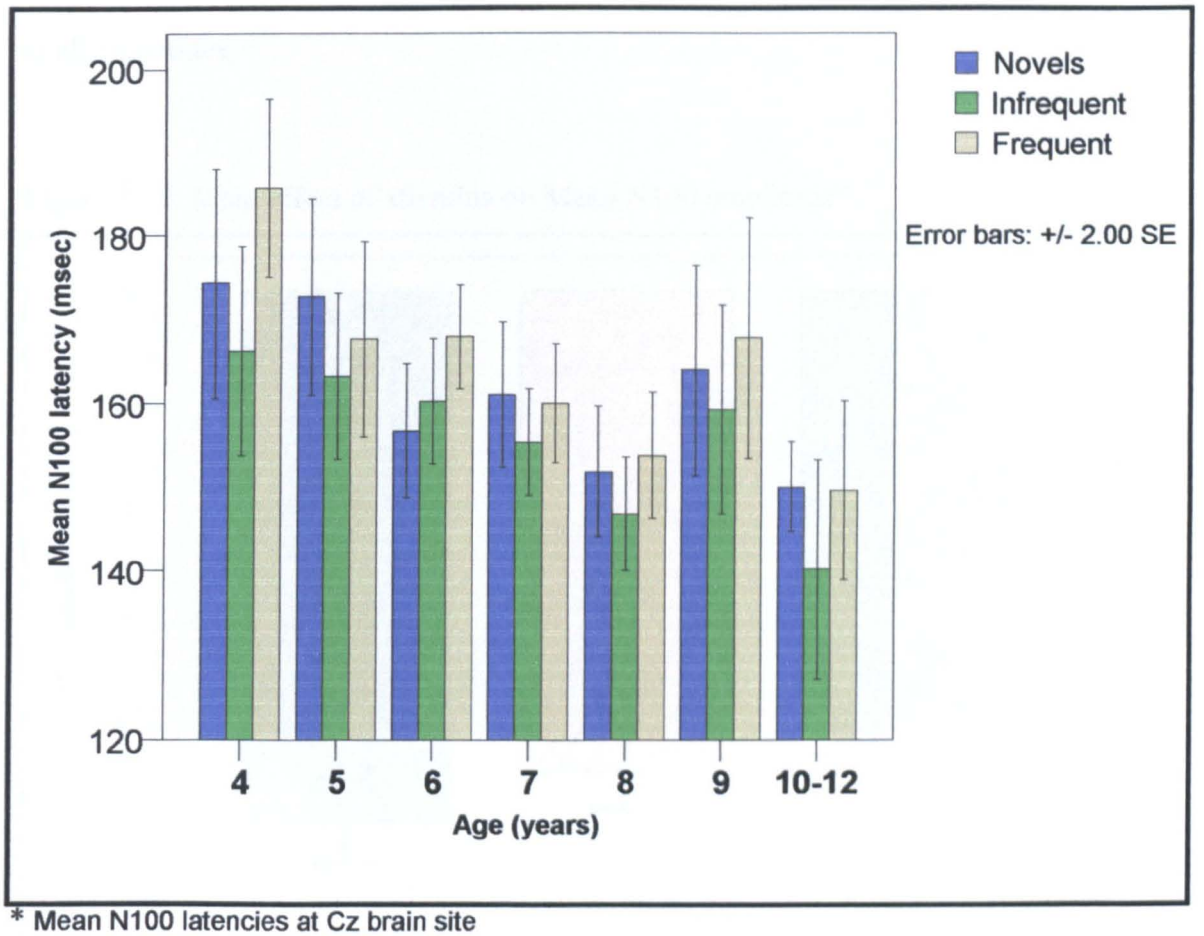
* Average ERP waveforms to novel visual stimuli at frontal brain site (Fz)

5.4.2.1 Visual N100

Latency:

Analyses of the N100 latency revealed a significant main effect of Stimulus [$F(2, 316) = 8.013$ ($p < 0.001$)], Electrode [$F(3, 474) = 70.559$ ($p < 0.001$)] and Age [$F(6, 158) = 4.260$ ($p < 0.001$)]. *Post hoc* analyses showed that the N100 latency of the infrequent stimulus was significantly shorter than that of the frequent stimulus showing that the children reacted faster to the infrequent stimulus. The main effect of Electrode occurred because the latency of N100 at Pz was significantly longer than those of the other fronto-central electrodes. The main effect of Age occurred due to decrease in N100 latency by age (figure 5.9).

Figure 5.9: Mean N100 latency by age*



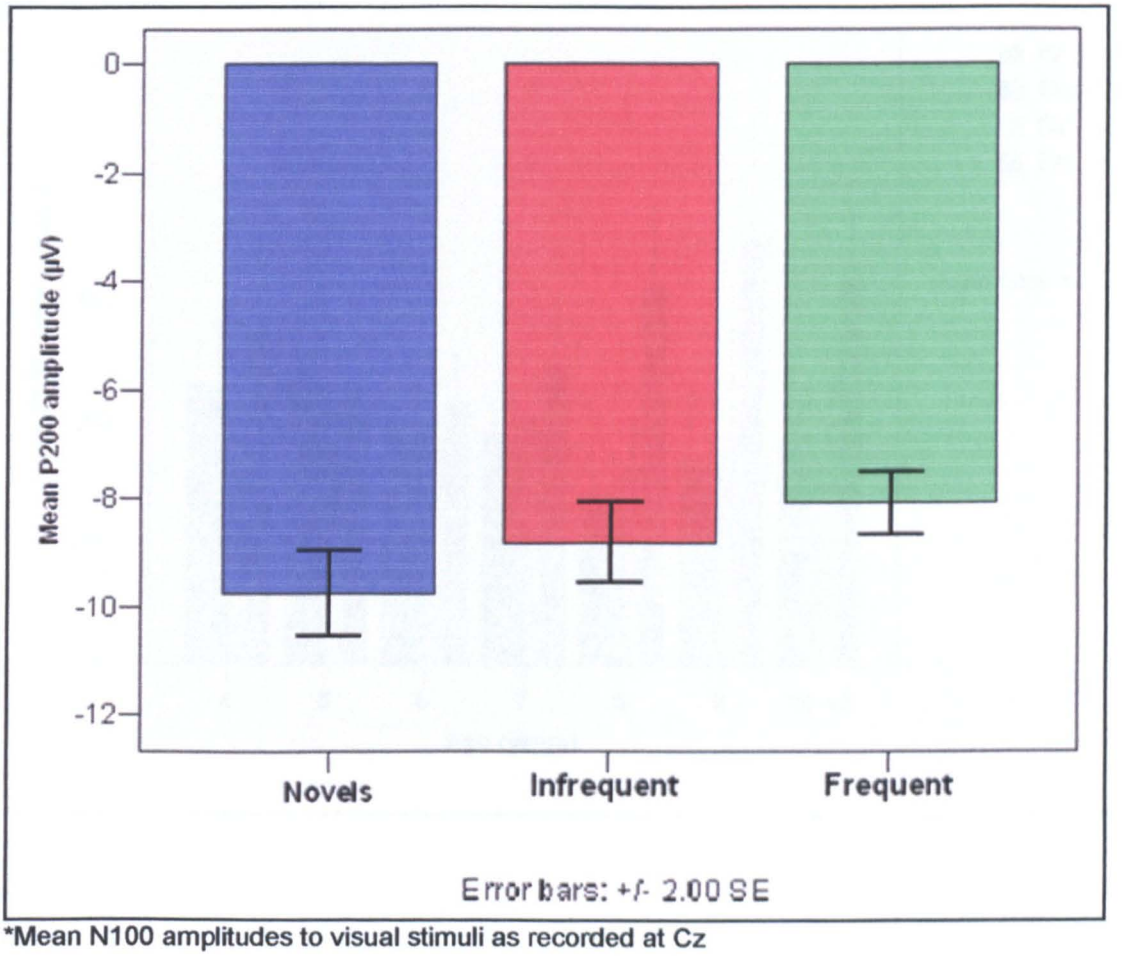
* Mean N100 latencies at Cz brain site

Amplitude:

There were significant main effects of Stimulus [F (2, 316) = 9.38 ($p < 0.001$)], Electrode [F (3, 474) = 14.435 ($p < 0.001$)] and Age [F (6, 158) = 2.489 ($p = 0.025$)] and interactions of Stimulus by Electrode [F (6, 948) = 8.989 ($p < 0.001$)] on N100 amplitude. *Post hoc* analyses revealed that the main effect of stimulus was due to significantly larger N100 amplitude to novel than frequent stimuli (Figure 5.10). The main effect of electrode occurred because the N100 amplitude at Pz was significantly smaller than at the fronto-central electrodes. There was a gradual decrease of N100 amplitude with age between 6 – 9 years. The interaction of Stimulus by Electrode occurred because the amplitude to the novel images was greater at the fronto-central electrodes than posterior electrodes. *Post hoc* analysis

did not reveal a significant Electrode by Age effect on the N100 amplitude by age at all electrodes.

Figure 5.10: Main effect of stimulus on Mean N100 amplitude*



*Mean N100 amplitudes to visual stimuli as recorded at Cz

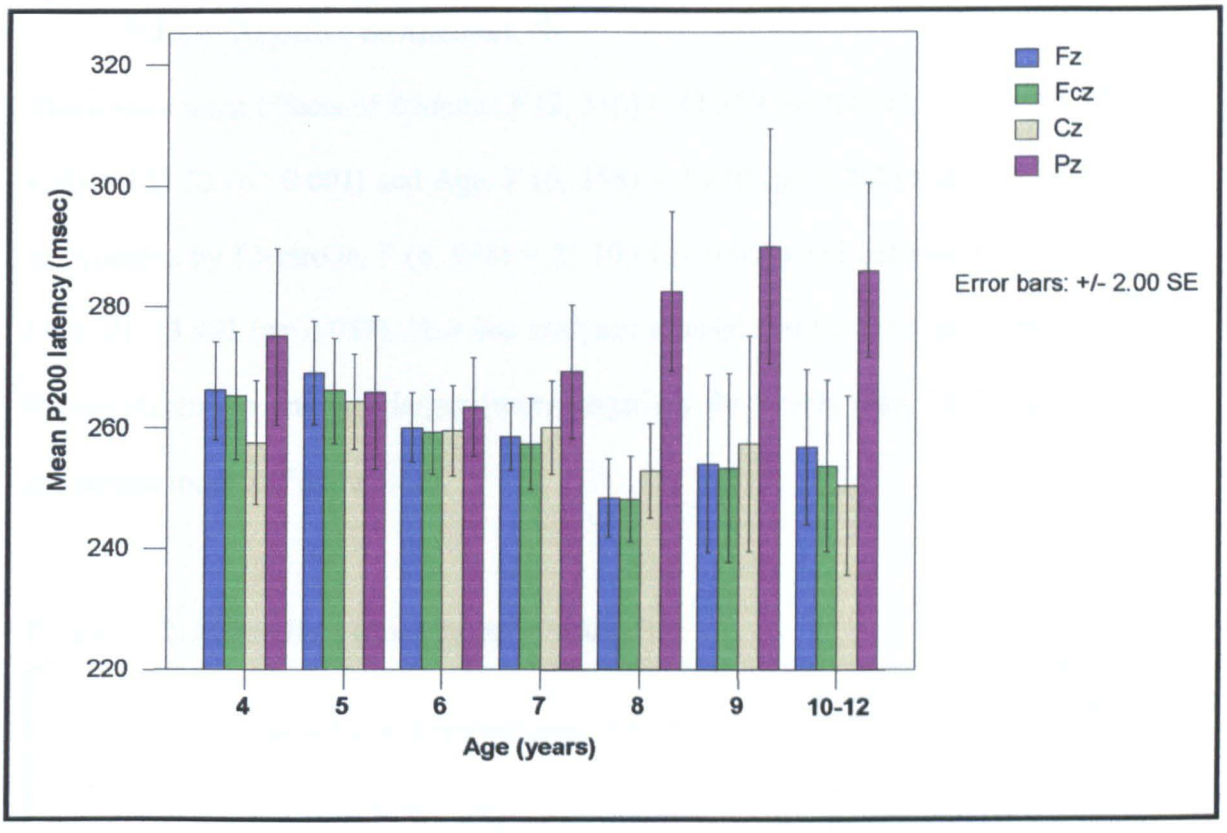
5.4.2.2 Visual P200

Latency:

There were main effects of both Stimulus [F (2, 316) = 8.868 ($p < 0.001$)] and Electrode F [(3, 474) = 20.52 ($p < 0.001$)] and an interaction of Electrode by Age [F (18, 474) = 3.592 ($p<0.001$)] on P200 latency. *Post hoc* analyses revealed shorter latencies to the novel than frequent image. The P200 latency was significantly longer at Pz location compared to the other fronto-central electrodes. The Electrode

by Age interaction occurred because the mean P200 latency at Pz was significantly longer than at the fronto-central electrodes from 7-10 years (Figure 5.11).

Figure 5.11: Age as a between subjects factoring P200 latency



Amplitude:

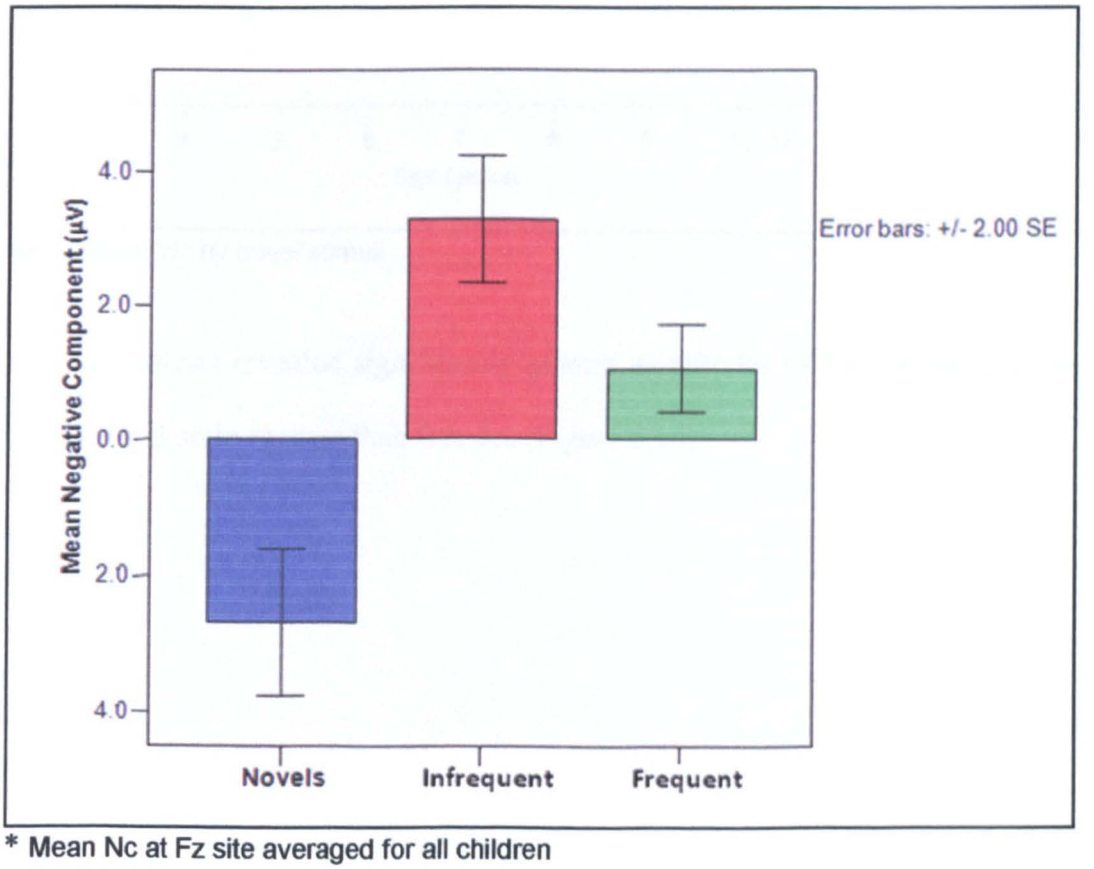
There were main effects of Stimulus F [(2, 316) = 45.03 ($p < 0.001$)], Electrode [F (3, 474) = 4.72 $p = 0.021$] and Age [F (6, 158) = 4.534 ($p < 0.001$)] and a significant interaction effect of Stimulus by Electrode [F (6, 948) = 16.424 ($p < 0.001$)] on P200 amplitude. The main effect of stimulus occurred because the P200 amplitude for the novel visual stimuli was significantly smaller than that for the frequent or infrequent stimulus. The amplitudes were largest frontally and decreased progressively posteriorly. The main effect of Age occurred due to a significant increase of amplitude with increasing age. The interaction of Stimulus by Electrode

occurred because the amplitude was greater at the fronto-central electrodes than posterior location for the frequent and infrequent images. The P200 amplitude increased between 4-7 years and then decreased between 9-10 years.

5.4.2.3 Negative component, Nc

There were main effects of Stimulus F (2, 316) = 42.559 ($p < 0.001$), Electrode F (3, 474) = 133.53 ($p < 0.001$) and Age, F (6, 158) = 3.810 ($p = 0.001$) and interactions of Stimulus by Electrode, F (6, 948) = 21.109 ($p < 0.001$) and Electrode by Sex, F (3, 474) = 3.491 ($p = 0.047$). *Post hoc* analyses showed that the Nc was largest over frontal electrodes and was larger (more negative) for novels than the frequent or infrequent images (Figure 5.12).

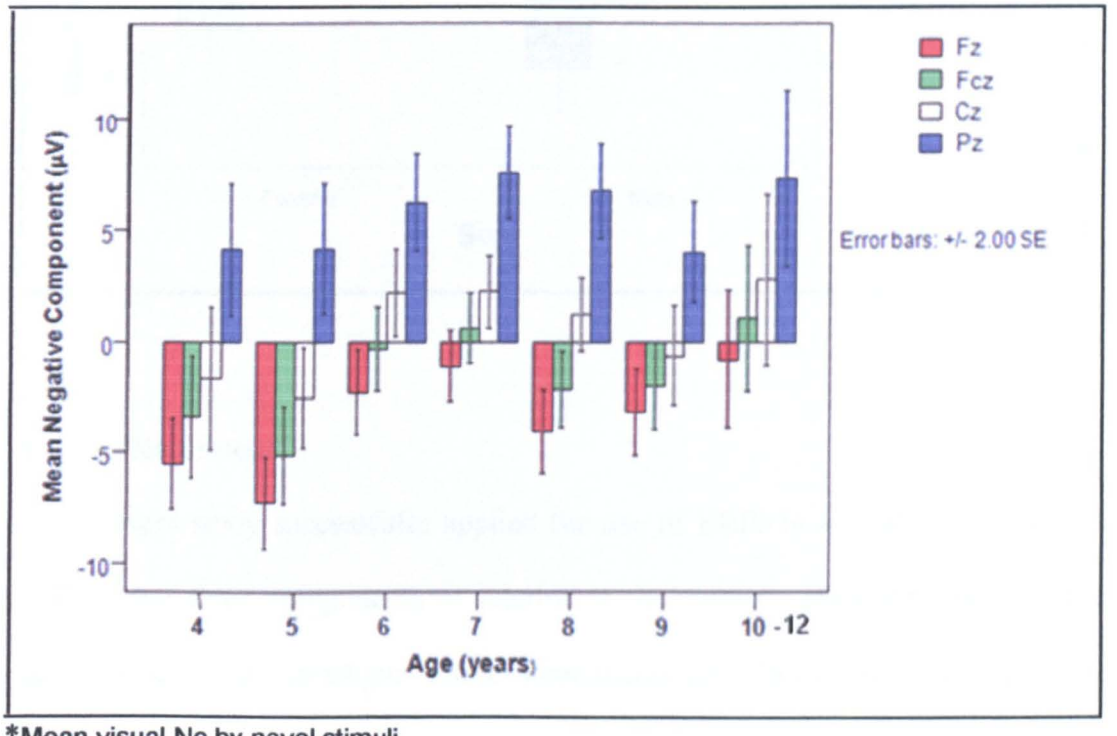
Figure 5.12: Main effect of stimulus on visual Nc*



* Mean Nc at Fz site averaged for all children

The interaction of Stimulus by Electrode occurred because the effect of stimulus was significant at fronto-central but not parietal electrodes. The main effect of Age occurred due to decrease in the Nc amplitude with increasing age except for ages 8 and 9 (Figure 5.13).

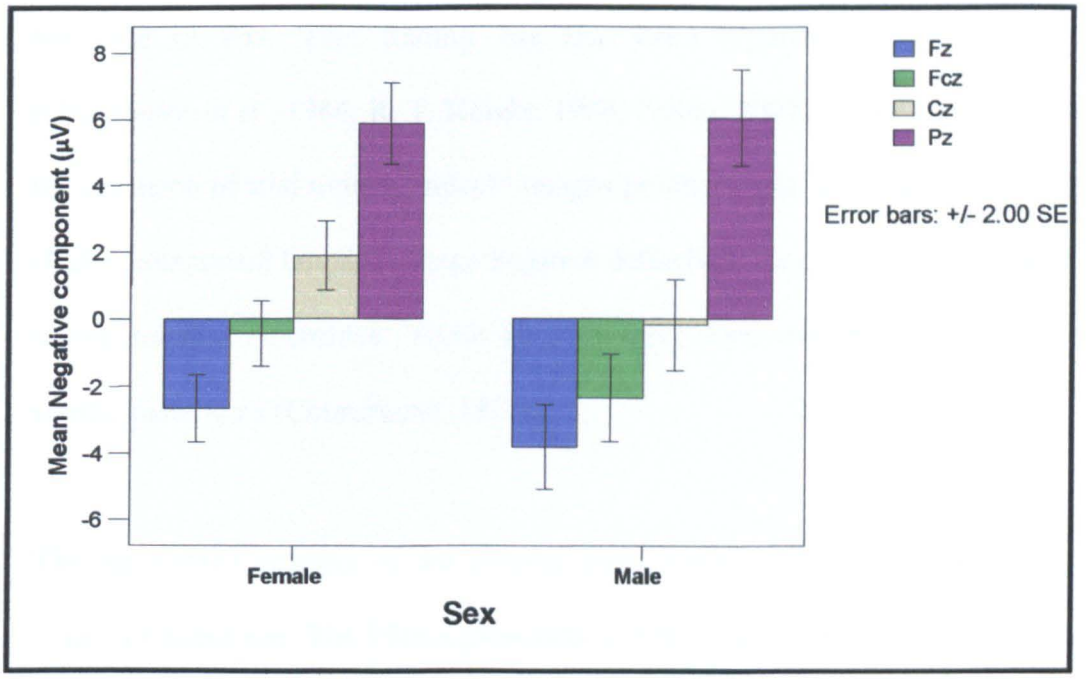
Figure 5.13: Effect of Age by electrode interaction on mean Nc amplitude*



*Mean visual Nc by novel stimuli

Post hoc analysis revealed significantly greater amplitudes of Nc for males at the fronto-central brain regions than females (Figure 5.14).

Figure 5.14: Negative component: Electrode by Sex



5.5. Discussion:

The present study successfully applied the use of ERPs in a rural area of Kenya. There were three components of interest in the auditory paradigm and a further three in the visual paradigm. These components have been observed in previous studies that used similar experimental conditions mainly the same inter-stimulus interval (Albrecht, Suchodoletz, & Uwer, 2000; Bishop, Hardiman, Uwer, & von Suchodoletz, 2007; Ceponiene et al., 1998; Ceponiene, Yaguchi et al., 2002; Ponton et al., 2000) and are thought to represent perceptual-cognitive mechanisms. The present study examines the latencies and amplitudes of P100, N200 and P3a components in the auditory and N100, P200 and Nc components in the visual paradigm, of children aged 4-12 years.

In the auditory paradigm, this study demonstrated that the presence of unique environmental sounds (novel stimuli), which were interspersed among frequent and

infrequent tones, elicited a positive component with a fronto-central maximum at the time of P3a. This finding has also been reported by previous studies (Courchesne et al., 1984; R. T. Knight, 1984; Polich, 2007). In the visual paradigm, the inclusion of trial unique "novel" images produced not only the N100 and P200 (P250) component but also a large negative deflection (Nc) with a frontal maximum at the midline electrodes. These findings have been reported elsewhere using similar paradigms (Courchesne, 1978).

The age-related changes in the present study included decrease of P100 latency with increasing age. The P100 component is interpreted as a marker of preferential attention and thought to reflect the level of arousal (Key et al., 2005). There was also decrease in P100 amplitude with increasing age between 7-12 years. The maturation effects of auditory ERPs were studied in 118 subjects aged between 5-20 years old (Ponton et al., 2000). The results showed that the mean P100 latency decreased with age between 5-12 years. In the same age group, there was an initial increase in amplitude between 5-8 years followed by a decrease between 9-12 years. In the visual paradigm of the present study, increase in age was associated with decrease in N100 latency and N100 amplitude. As for the visual P200, there was an initial increase in amplitude between 5-7 years that then decreased between 9-10 years. In the present study, the negative component, Nc decreased with age. This finding has been shown in previous studies (Courchesne, 1978). The Nc is interpreted to reflect a child's allocation of attention, with negativity to infrequent stimuli indexing orientation towards the novel (Courchesne, 1978).

The sex-related differences in the present study were significant associated with the visual P200 latency. The results showed that the P200 latency of males was significantly shorter than for females. While this might suggest that the males reacted faster than females in this group, there are no previous studies with similar findings. The percentage of males who were school-going was not significantly different from that of females and hence we cannot attribute latency differences to schooling.

The present study also included socioeconomic status, nutrition, head circumference and schooling in the model as between-subjects factor to determine their effects on the ERP components. These independent variables did not show any consistent influence on the ERP components in the present study. This is in contrast with neuropsychological tests that are greatly influenced by schooling, malnutrition and SES (Holding et al., 2004). We may speculate that the use of a proxy for SES (mother's education) may have diminished the effect of SES. However, in resource poor settings, mother education remains the best predictor of SES. Most children in the control group only had nursery school education and hence the effect of schooling may have been diminished. Head circumference was associated with age but there was consistent association with the ERP components. In previous studies, especially of autistic children, it has been found to correlate positively with cognition.

In conclusion, ERPs can be measured in children living in rural Kenya. The components are influenced by age, and to a less extent sex but not SES, nutrition and schooling. Since passive ERPs are devoid of language barriers and assessor-

subject interaction problems, they may be a better indicator of neural functioning in children.

CHAPTER 6

The effect of severe falciparum on cognition as measured by Event Related Potentials

6.1. Introduction

Plasmodium falciparum is the most common parasitic infection of the central nervous system (CNS), with over 500 million people exposed to the infection and accounting for over 1 million deaths per year (Snow et al., 2005). Children under 5 years old, living in Sub-Saharan Africa, bear the brunt of the disease whose most common neurological complications are seizures and cerebral malaria (CM) (Newton & Warrell, 1998). These neurological manifestations are associated with neurocognitive sequelae (see chapter 1).

The estimation of the burden of neurocognitive sequelae has been hampered by the lack of appropriate tools in malaria endemic areas. The high rate of illiteracy amongst poor rural populations, lack of familiarity with test demands and the fact that children rarely interact with adult strangers complicates the use of neuropsychological tests in rural areas (Holding et al., 2004). The use of adapted neuropsychological tests, rather than tests developed for local populations has been thought to introduce test bias in their reporting and interpretation (Carter, Mung'ala-Odera et al., 2005; Kihara et al., 2006). In view of these considerations, I examined brain processing as measured with event related potentials (ERPs) to assess the cognition in children exposed to severe falciparum malaria in rural Kenya.

6.1.1 Pathophysiology of falciparum malaria

The pathophysiology of severe malaria is complex and poorly understood (White & Ho, 1992). *Plasmodium falciparum* is a parasite whose development within the red blood cells causes symptoms in humans. The infected red blood cells, in the later stages, become sequestered within the vasculature of the brain, but do not invade the parenchyma of the brain. The sequestration occurs in both the gray and white matter throughout the brain, giving rise to a diffuse encephalopathy.

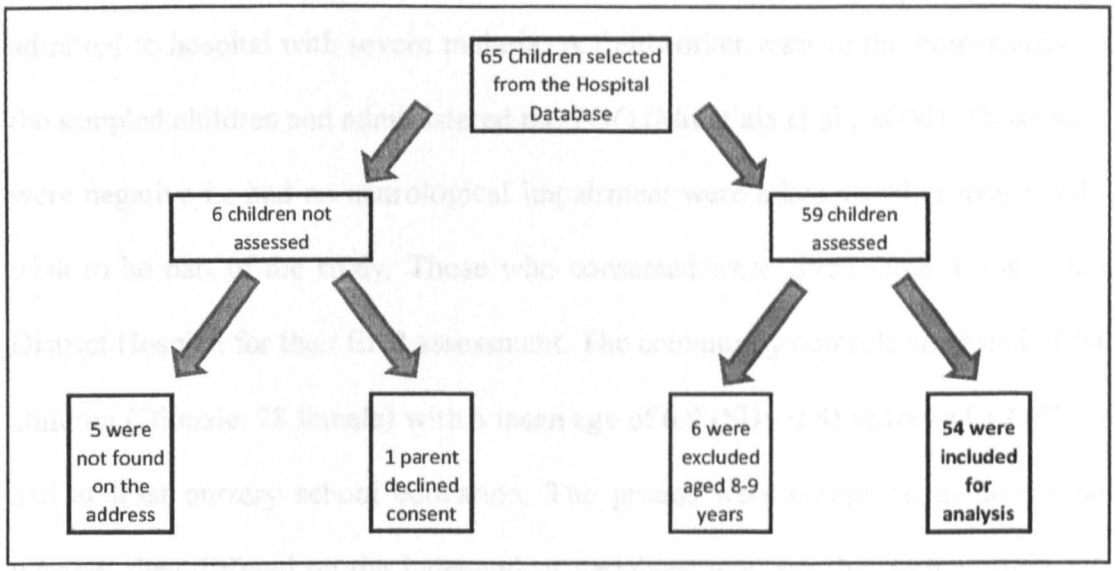
There is a growing body of evidence to suggest that neurocognitive sequelae do occur post severe malaria, in particular children with complicated seizures (i.e. focal, repetitive or prolonged) (Carter, Mung'ala-Odera et al., 2005; Carter, Neville et al., 2003; Holding et al., 1999).

6.2. Methods

6.2.1 *Subjects*

This was a study involving children admitted to Kilifi District Hospital (KDH) with a history of severe falciparum malaria. The study was conducted between July 2004 and September 2006. Sixty-five children aged 6-9 years old and admitted to KDH between May 2002 and March 2004 with severe falciparum malaria were selected. Five children could not be traced from the homestead description and one parent declined consent. There were only 5 children between 8-9 year age-group and these were subsequently dropped from analysis due to small number (Figure 6.1).

Figure 6.1: Flowchart showing recruitment of children with severe malaria



Fifty-four children aged 6-7 years were analyzed but four were subsequently dropped since they didn't meet the study criteria (3 were found to have had encephalitis and 1 had meningitis). The results of fifty children (23 male; 27 female) aged between 6-7 years old were analyzed (Table 6.1). Their mean age was 6.9 (SD= 0.7) years and 31 (63%) of them had enrolled at least to nursery school. The parents reported the children's most preferred hand; forty-six children (93%) were right-handed.

Table 6.1: Children sampled from KDH database and community controls

Diagnosis	Age of the Children		Female (%)	Schooling (%)
	6 years	7 years		
Cerebral Malaria	22	5	15 (56%)	18 (66.7%)
Malaria plus seizures	9	5	7 (50%)	8 (57.1%)
Prostrate Malaria	8	1	5 (56%)	6 (66.7%)
Control group	40	14	28 (52%)	39 (72.2%)
Total	79	25	55 (53%)	71 (68%)

An age-matched control group was selected from the EPI-DSS who had not been admitted to hospital with severe malaria. A fieldworker went to the homesteads of the sampled children and administered the TQQ (Mung'ala et al., 2004). Those who were negative i.e had no neurological impairment were asked whether they would wish to be part of the study. Those who consented were given fares to the Kilifi District Hospital for their ERP assessment. The community controls consisted of 54 children (26 male; 28 female) with a mean age of 6.9 (SD= 0.8) years and 39 (72%) had at least nursery school education. The groups were compared to determine whether they differed on the independent variables; age, sex, head-circumference, schooling and SES. One-way analysis of variance showed no difference between the groups on all these independent variables (Table 6.2).

Table 6.2: Comparison between children with exposure to severe malaria and controls on independent variables

	CT (n= 54) mean± SD	CM (n= 27) mean± SD	MS (n= 14) mean± SD	PM (n= 9) mean± SD	F	p-value
OFC* (cm)	49.95 ± 1.5	50.58 ± 1.9	50.00 ± 2.2	50.90 ± 1.6	1.339	0.266
Age (yrs)	6.3 ± 0.4	6.2 ± 0.4	6.4 ± 0.5	6.1 ± 0.3	0.796	0.499
Sex (%Male)	48%	44%	50%	44%	0.056	0.983
Schooling*	72%	67%	57%	67%	0.799	0.498
SES*	14%	27%	21%	11%	0.400	0.754

* OFC represents the occipito-frontal head-circumference, Schooling represents children attending at least nursery school, SES represents mothers with at least 3 years of basic education

Children with gross neurological dysfunctions were to be excluded from the study as were those whose parent(s) denied consent. In the present study, no child was excluded due to gross neurological dysfunction. Auditory and visual screening was carried out using Kamplex audiometer and Sonksen-Silver tests respectively and all

the children were within acceptable thresholds. The assessment of children exposed to severe falciparum malaria and community controls took place simultaneously and my assistants and I were blinded to the group status of the children.

6.2.2 Measurement of the ERPs and analysis

Auditory and visual ERPs were recorded from 18 scalp electrodes (Ag/AgCl) using standard 10-20 system. Continuous EEG data were recorded at a sampling rate of 500 Hz and low-pass filtered offline at 20 Hz, and divided into epochs according to stimulus presentation at -200 to 1000 msec for auditory and -200 to 1500 msec for visual stimuli. A detailed description of the methods and data analysis is given in Chapter 4 of the thesis.

6.3. Auditory ERP Results

The amplitudes and latencies of each of the ERP components are shown in table 7.2a/b. The latencies of N200 at Fz, Fcz, Cz and Pz were significantly longer in those of children exposed to CM, M/S or PM compared to the controls (Table 6.3a). The P3a latencies were significantly longer for children exposed to CM and M/S than those of controls. There was no difference in the latency of the P100 between any of the malaria groups and the controls. The only significant difference detected in the amplitudes was over the parietal (Pz) electrode in the CM group compared to controls and over the fronto-central region in the M/S group (Table 6.3b).

Table 6.3a: Comparison of mean latencies to novel auditory stimuli to control group

		Latency (msec)							
		CM (n=27)		M/S (n=14)		PM (n=9)		Control (n=54)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
P100	Fz	91.2	25	92.5	25.8	100.6	17.3	98.3	29.4
	Fcz	92.9	27.8	89	25	100.8	16.8	99.8	29.8
	Cz	91.8	29	84	16.2	97.3	17.8	97.1	26.1
	Pz	93.7	29	80.7	30.7	101.3	21	97.9	28.7
N200	Fz	**252.5****	47.7	**272.1****	66	**302.4****	57.4	182.6	41.5
	Fcz	**251.6****	49.6	**263.7****	58.2	**280.8****	62.5	179.5	42.0
	Cz	**247.3****	52.1	**267.2****	57.3	**277.5****	55.5	174.5	42.6
	Pz	**250.4****	50.7	**277.0****	49.2	**259.5****	45.2	185.1	38.2
P3a	Fz	331.4	56.9	326.1	61.2	343.7	55.6	308.2	59.1
	Fcz	**329.7***	55	337.8	58.1	333.5	57.7	304.6	51.8
	Cz	**326.5***	55.9	**334.2***	59.8	336	63.2	297.8	57.6
	Pz	**347.6***	48.3	351	78.8	335.3	43.3	313.6	63.3

The difference was determined by means of independent samples 2-tailed t-test: *P< 0.05; **P< 0.01

Table 6.3b: Comparison of mean amplitudes to novel auditory stimuli to control group

		Amplitude (μV)							
		CM (n=27)		**M/S (n=14)**		**PM (n=9)**		**Control (n=54)**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
P100	**Fz**	10.6	5.7	11.4	6.3	9	3.3	10.4	6.3
	Fcz	10.2	5.5	10.8	7.3	9.5	4.6	10.3	5.7
	Cz	8.6	4.8	8.9	6.3	8.1	5.5	9.4	5.4
	Pz	3.1	4.7	3.7	3.5	6.3	5.7	4.2	4.7
N200	**Fz**	-4.4	7.1	-1	10.6	0.8	6.5	-3.9	9.9
	Fcz	-1.6	7	0.9	10.4	3.5	7.5	-1.8	8.9
	Cz	-2.7	8.2	-0.6	9.8	1.9	6.6	-1	8
	Pz	**-8.8***	8.1	-6.8	10.9	-1.8	4.4	-5.2	7
P3a	**Fz**	5.30	8.6	2.8	13.7	4.40	6	7.5	10.2
	Fcz	9.00	8.1	**5.9***	11.2	7.80	9.6	11.3	9.8
	Cz	6.20	7.3	5.7	10.2	6.20	9.3	10.2	9.4
	Pz	**0.2****	5.9	2.9	10.1	3.80	6.4	6.2	7.7

The difference was determined by means of independent samples 2-tailed Mann-Whitney U test: *P< 0.05

6.3.1 AUDITORY P100

Latency: The P100 component revealed a significant main effect of Diagnosis [F (3, 89) = 2.685, p= 0.05]. *Post hoc* analyses did not reveal any significant difference between the P100 latencies depending on group status either exposed or unexposed to severe malaria.

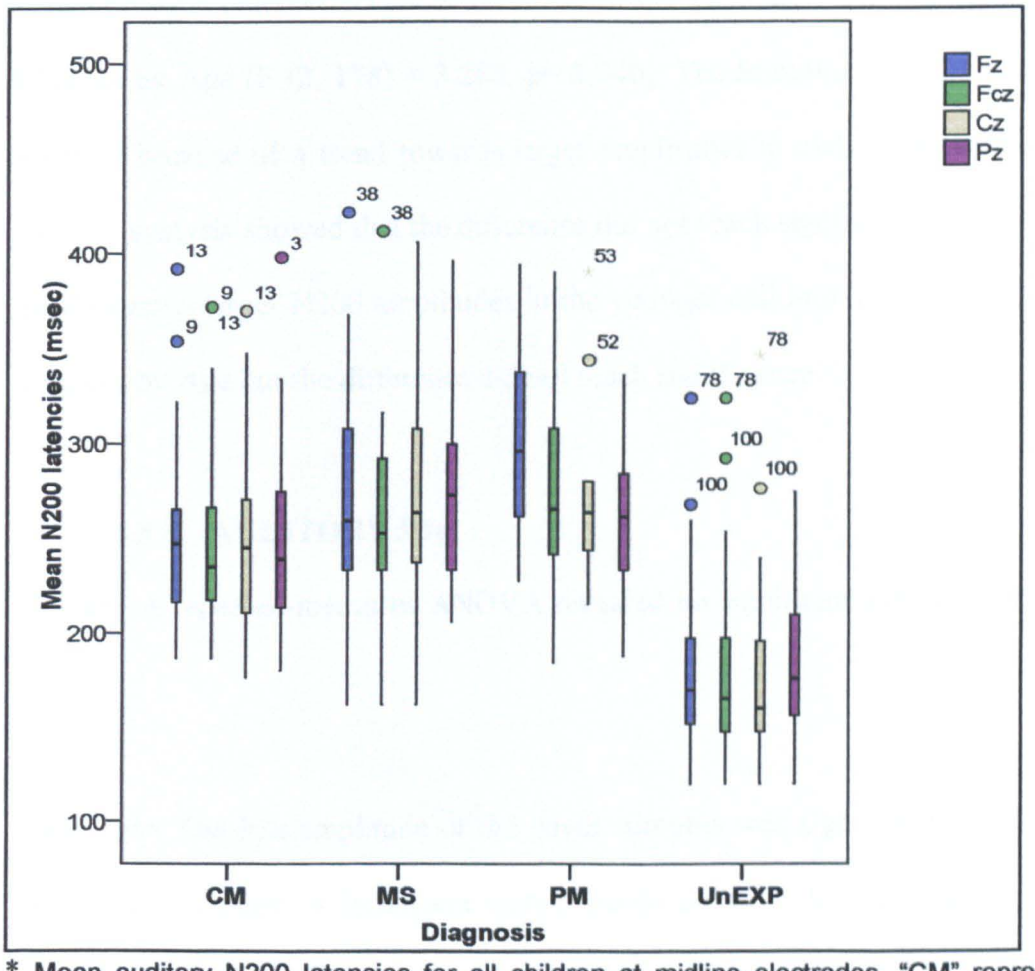
Amplitude: In the amplitude for the P100 component, there was a significant main effect for Electrode [F (3, 267) = 83.964, p< 0.001]. *Post-hoc* analysis showed that the P100 amplitude had a front-central maximum. Its amplitude was significantly larger in the fronto-central electrodes than at Pz. The results also showed a significant interaction effect of Stimulus by Age by Sex [F (2, 178) = 3.87, p= 0.024]. *Post hoc* analysis did not show any significant differences in stimulus response based on Age and Sex.

6.3.2 AUDITORY N200

Latency: The latency of the N200 component revealed a significant main effect of Stimulus [F (2, 178) = 6.004, p= 0.007] and Diagnosis [F (3, 89) = 11.235, p< 0.001]. There were significant interaction effects of Stimulus by Diagnosis [F (6, 178) = 17.89, p< 0.001], Stimulus by Electrode [F (6, 534) = 2.605, p= 0.044]. *Post-hoc* analysis showed that the N200 latency to the infrequent and novel stimuli was significantly shorter than that of the frequent stimulus indicating that the children reacted faster to the novel and infrequent stimulus. The main effect of Diagnosis occurred because children exposed to severe malaria had significantly longer N200 latencies compared to community controls (shown in Table 6.2a previously). The significant interaction of Stimulus by Diagnosis occurred because

the N200 latency to the novel stimulus was significantly longer for children with a history of CM, M/S and PM than study controls (Figure 6.2) but it was not true for the other stimuli. The Stimulus by Electrode interaction occurred because the latency of N200 to the novel stimuli was significantly shorter than the frequent stimuli at Cz and Fcz showing that the children reacted faster to the novels at the frontal-central sites.

Figure 6.2: Boxplot showing mean N200 latencies by diagnosis



* Mean auditory N200 latencies for all children at midline electrodes. "CM" represents cerebral malaria, "MS" is malaria plus seizures, "PM" is prostrated malaria and "UnEXP" represents unexposed or control group

Amplitude: The N200 amplitude revealed a significant main effect of Stimulus [F (2, 178) = 35.807, p< 0.001] that occurred because the amplitude of the N200 of the novel stimulus was significantly smaller than that to the frequent or infrequent

stimuli. The diagnosis of the children influenced their mean N200 amplitude (main effect of Diagnosis [F (3, 89) = 3.302, p= 0.024]). Children with a history of PM and M/S had the smallest amplitudes (-2.6 and -4.9μV respectively), while children with a history of CM and controls had larger amplitudes (-7.9 and -7.5μV respectively). There was a significant interaction effect of Stimulus by Electrode [F (6, 534) = 25.34, p< 0.001] that occurred due to significantly larger amplitude to the infrequent stimulus than novel at the fronto-central sites (Fz, Fcz and Cz). There were other interaction effects of Stimulus by Sex [F (2, 178) = 3.231, p= 0.043] and Stimulus by Age [F (2, 178) = 3.288, p= 0.040]. The Stimulus by Sex interaction occurred because of a trend towards larger amplitudes in males than females but *post hoc* analysis showed that the difference did not reach significance. There was a trend towards larger N200 amplitudes in the younger children in the interaction of Stimulus by Age but the difference did not reach significance.

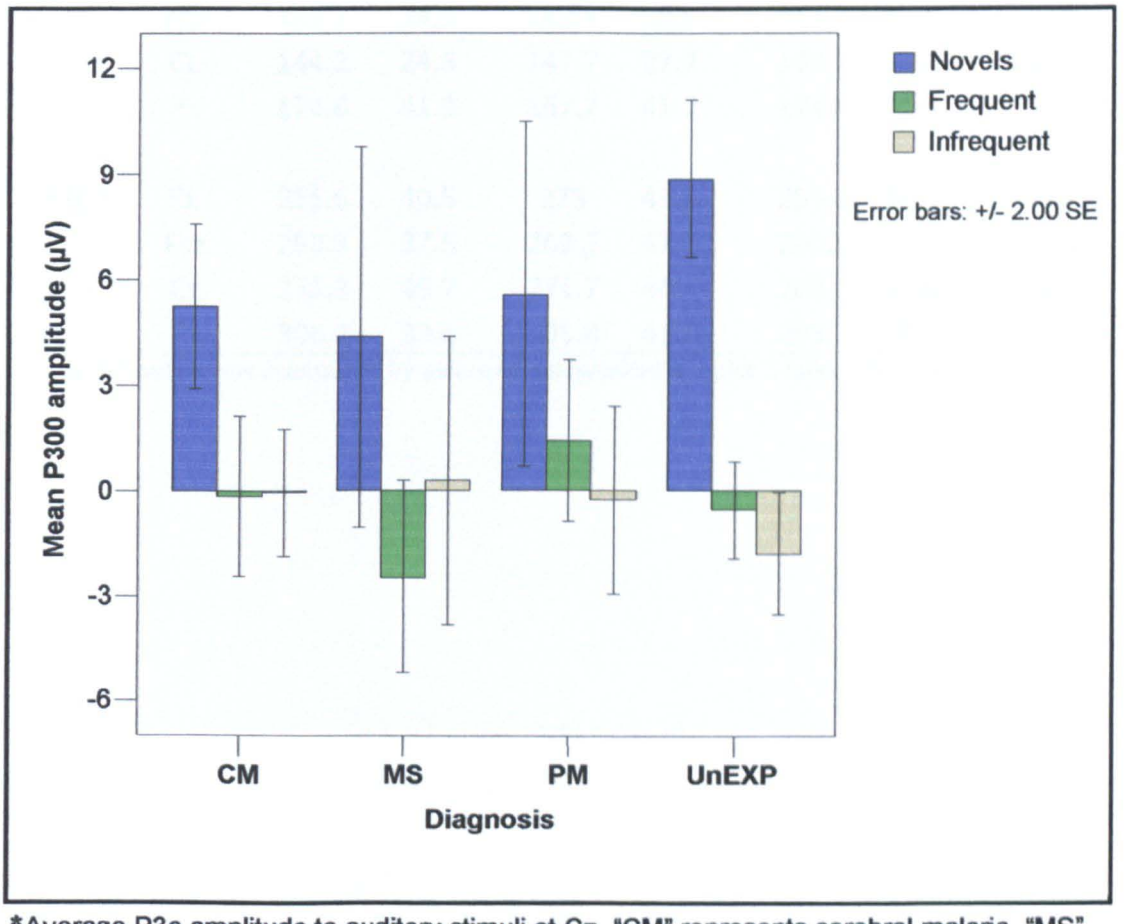
6.3.3 AUDITORY P3a

Latency: A repeated-measures ANOVA revealed no significant effects for the P3a latency.

Amplitude: The P3a amplitude of the novel stimulus was significantly larger than that of the frequent or infrequent stimuli (main effect of Stimulus [F (2, 178) = 30.127, p< 0.001]). There was an effect of electrode due to larger P3a amplitude at the Fcz and Cz electrodes [F (3, 267) = 7.851, p< 0.001]. The results showed that the P3a amplitude was larger over the fronto-central region. There were also significant interaction effects of Stimulus by Diagnosis [F (6, 178) = 2.504, p= 0.026] due to smaller P3a amplitudes in exposed children than community controls

(Figure 6.3). There were interactions of Stimulus by Age [F (2, 178) = 3.578, p= 0.033] and Stimulus by Electrode [F (6, 534) = 7.684 (p< 0.001)]. The significant interaction of Stimulus by Age occurred due to the older children having significantly larger P3a amplitude to the novel stimulus than the younger ones. The interaction of Stimulus by Electrode was due to significantly larger amplitude to the novel stimuli at all mid-line brain locations (i.e. Fz, Fcz, Cz and Pz).

Figure 6.3: P3a amplitude: Stimulus by Diagnosis*



*Average P3a amplitude to auditory stimuli at Cz. "CM" represents cerebral malaria, "MS" is malaria plus seizures, "PM" is prostrated malaria and "UnEXP" represents unexposed or control group

6.4. Visual ERP results

The amplitudes and latencies of each of the visual ERP components under investigation are shown in table 6.3a/b. The latencies of N100 and P200

components did not reveal significant differences between the groups (Table 6.4a) but the P200 amplitude and negative component, Nc of the children with a history of M/S showed significant differences (Table 6.4b).

Table 6.4a: Mean N100 and P200 latencies by diagnosis

		Latency (msec)							
		CM (n=27)		M/S (n=14)		PM (n=7)		Control (n=54)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
N100	Fz	146.1	23.9	144.5	32.5	146.4	19.7	151	32.6
	Fcz	142.7	24.3	142.4	28.6	144.6	19.8	154.2	33.7
	Cz	144.2	24.8	147.7	27.7	139.7	20.4	155.3	34.7
	Pz	174.6	41.9	167.7	41.2	174.4	34.3	173.5	47.5
P200	Fz	255.6	40.5	273	43.8	259.1	41.6	264.6	43
	Fcz	253.3	37.5	269.2	47.6	260.8	53.5	260.3	44.8
	Cz	271.3	46.7	271.7	46.4	269.3	53.8	263	48.8
	Pz	306.2	39.6	301.8	41.6	295.7	57.1	295	65.5

The difference was determined by means of independent samples 2-tailed t-test: ***P< 0.05**

Table 6.4b: Mean N100 and P200 amplitudes by diagnosis

		Amplitude (μV)							
		CM (n=27)		M/S (n=14)		PM (n=7)		Control (n=54)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
N100	Fz	-11.6	4.3	-12.9	8	-12.3	6.6	-10.8	5.7
	Fcz	-11.9	5.8	-12.6	8.3	-10.8	5.1	-10.1	5.5
	Cz	-12.4	6.8	-14.1	9	-10.5	4.2	-10.1	5.1
	Pz	-7	7.7	-7.4	12.3	-6.6	4.8	-6.1	6.6
P200	Fz	5.3	8.0	**3.5***	15.4	2.0	6.3	6.1	8.2
	Fcz	3.5	7.8	**3.2***	15.8	2.0	5.8	5.5	7.6
	Cz	1.8	8.5	**-1.2***	10.1	1.0	5.0	4.5	7.0
	Pz	7.5	10.2	**3.7***	9.1	4.1	7.0	8.2	8.6
Nc	Fz	-7.8	8.9	-7.7	8.4	-8.7	6.7	-6.1	7.5
	Fcz	-6.2	9.5	-6.5	7.9	-6.0	5.4	-3.7	7.5
	Cz	-3.8	8.6	**-9.7****	10.1	-4.6	4.1	-1.9	7.9
	Pz	3.9	8.6	-0.4	7.1	**-1.3***	6.3	3.9	8.3

The difference was determined by means of independent samples 2-tailed Mann-Whitney U test: ***P< 0.05**

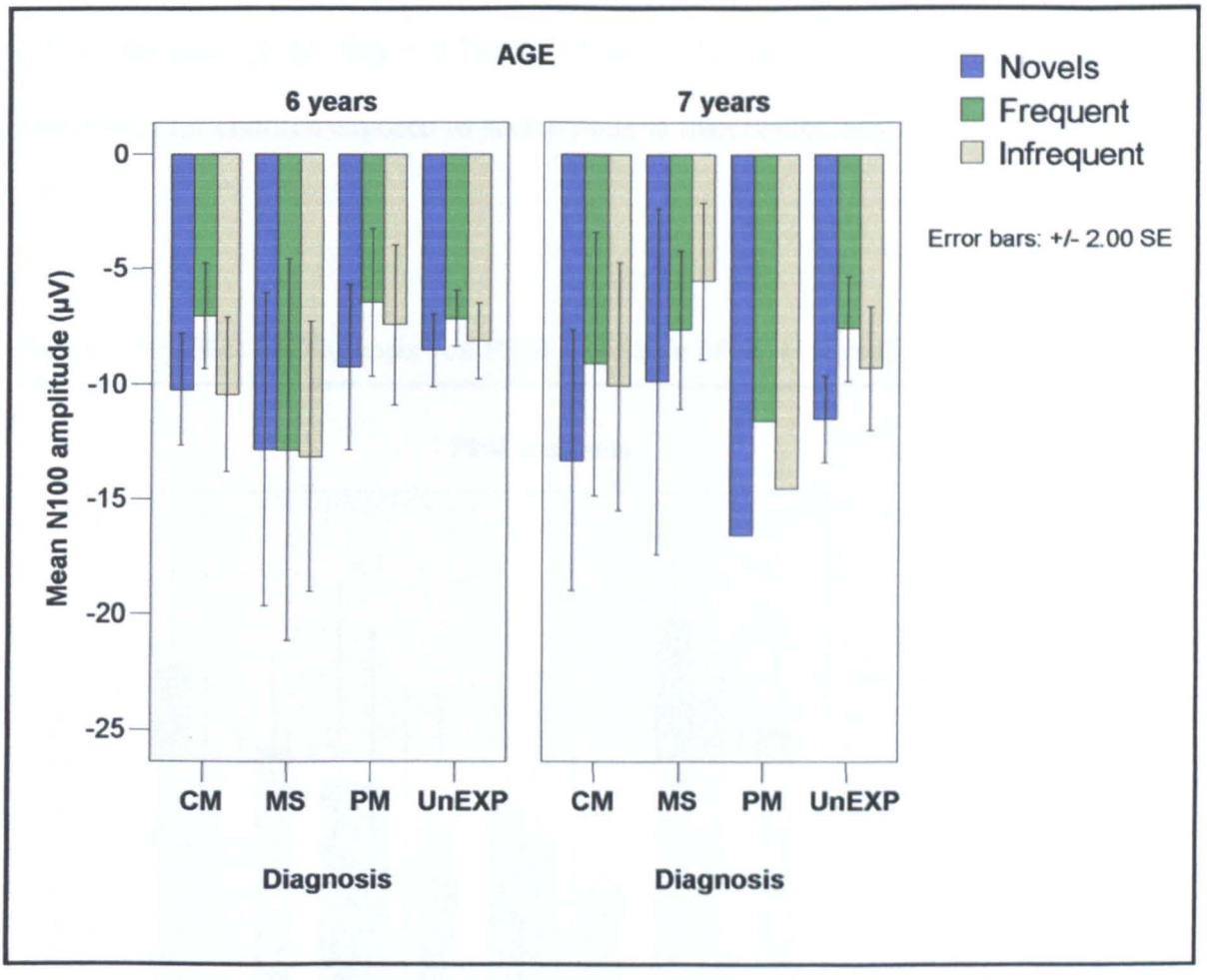
6.4.1 VISUAL N100

Latency: The analysis of the N100 latency using revealed a significant main effect of Stimulus [F (2, 178) = 4.698 (p= 0.018)] due to significantly shorter latencies to the infrequent and novel stimuli compared to the frequent. Thus children reacted faster to the infrequent and novel stimuli than the frequent. The latency of the N100 at Pz was significantly longer than the frontal and central electrodes (main effect of Electrode [F (3, 267) = 21.22, p< 0.001]). There was an interaction effect of Electrode by Diagnosis by Age [F (9, 267) = 2.447 (p=0.042)] but this interaction did not reveal any significant relationship upon *post hoc* analysis.

Amplitude: The amplitude of the N100 component to the novel and infrequent stimuli was significantly larger than the frequent stimulus (main effect of Stimulus

[F (2, 178) = 5.403, p= 0.006]). The amplitude was also significantly smaller at Pz compared to the fronto-central electrodes (main effect of Electrode, [F (3, 267) = 19.251, p< 0.001]). There were significant interaction effects of Stimulus by Electrode [F (6.534) = 4.459, p= 0.003] and Diagnosis by Age F (3, 89) = 2.7 (p= 0.05). The interaction of Stimulus by Electrode was due to larger amplitude of the N100 to the novel stimulus at Fz, Fcz and Cz. The N100 amplitudes of community controls, children exposed to CM and PM increased with increasing age but that of children with a history of M/S decreased with age, hence the interaction of Diagnosis by Age (Figure 6.4).

Figure 6.4: Interaction of Age by Diagnosis* in N100 amplitude



*"CM" represents cerebral malaria, "MS" is malaria plus seizures, "PM" is prostrated malaria and "UnEXP" represents unexposed or control group

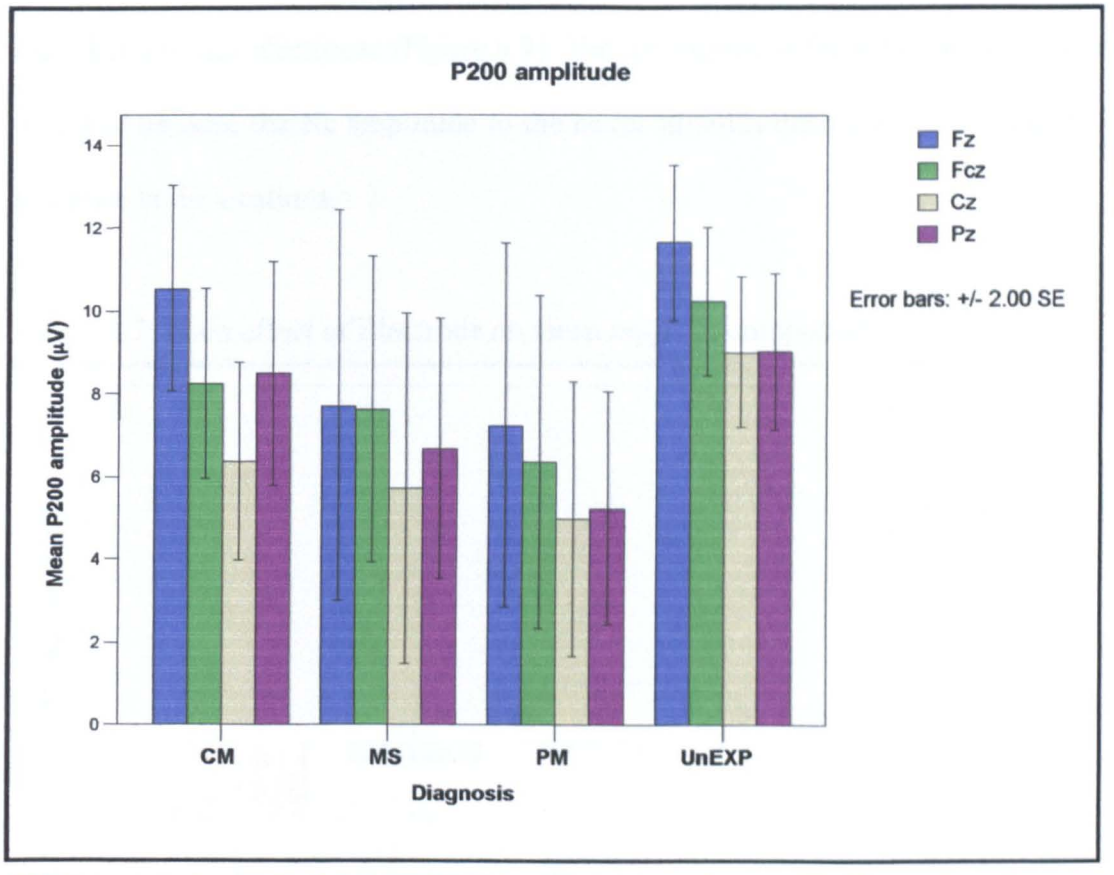
6.4.2 Visual P200

Latency: There was also a main effect of Electrode [F (3, 267) = 21.905 ($p < 0.001$)] due to significantly longer P200 latency at Pz compared to the fronto-central electrodes.

Amplitude: The P200 amplitude was significantly smaller for the novel stimulus compared to the frequent or infrequent stimuli (main effect of Stimulus [F (2, 178) = 16.49, $p < 0.001$]). There was a main effect of Electrode [F (3, 267) = 3.895 ($p = 0.038$)]. This was due to a trend towards larger P200 amplitude at Fz than the other

electrodes but it did not reach significance on *post hoc* analysis. There was a main effect Diagnosis [F (3, 89) = 2.788 (p= 0.045)] due to a trend towards smaller amplitudes for children exposed to severe malaria than community controls (Figure 6.5).

Figure 6.5: Effect of Diagnosis* on P200 amplitude of novel stimuli



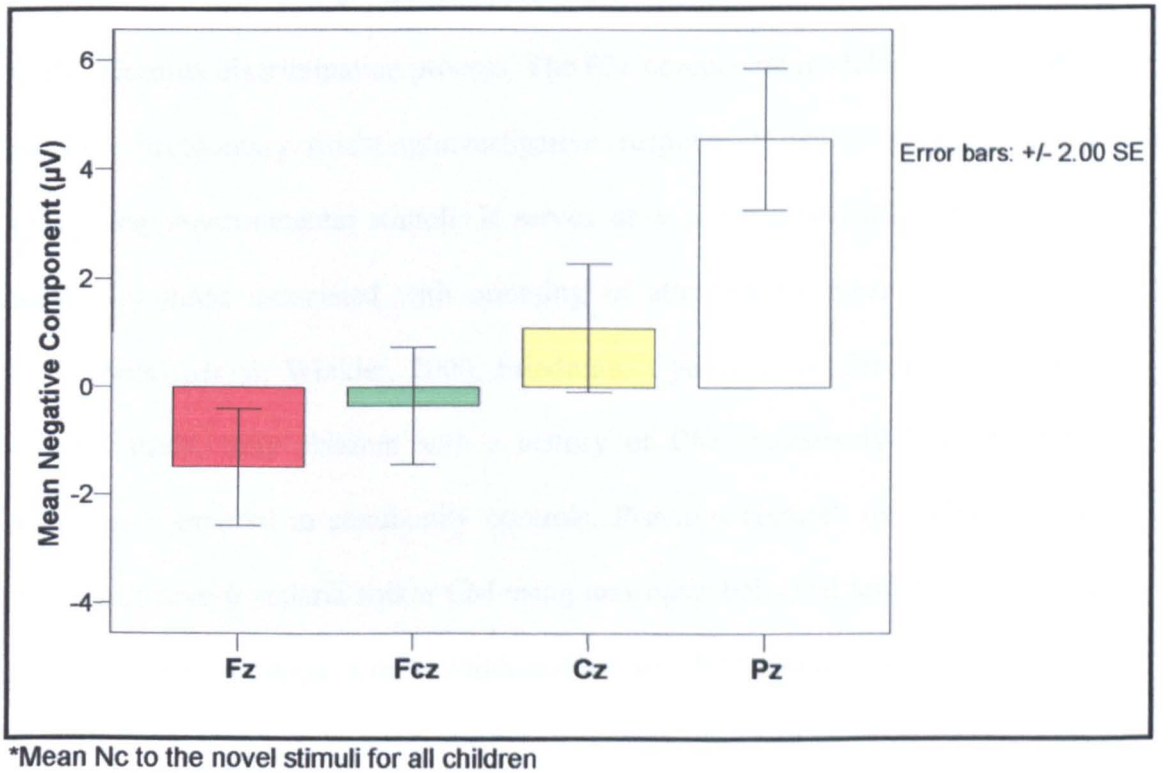
*"CM" represents cerebral malaria, "MS" is malaria plus seizures, "PM" is prostrated malaria and "UnEXP" represents unexposed or control group

There were significant interaction effects of Stimulus by Electrode [F (6, 534) = 6.719 (p< 0.001)] and Age by Sex [F (1, 89) = 4.672 (p= 0.033)]. The interaction between Stimulus and Electrode was due to significantly larger P200 amplitude to the infrequent stimuli than novel stimuli at all mid-line electrodes. The interaction of Age by Sex was not significant on post-hoc analysis.

6.4.3 Negative component, Nc

There was a significant main effect of Stimulus [F (2,178) = 24.522 ($p < 0.001$)] and Electrode [F (3, 267) = 22.664 ($p < 0.001$)] and a significant interaction of Stimulus by Electrode [F (6, 534) = 6.203 ($p < 0.001$)]. *Post hoc* analysis showed that the main effect of Stimulus was because the Nc was greatest by the novel stimulus. The main effect of Electrode was due to greater Nc amplitude at the frontal electrodes than the posterior electrodes (Figure 6.7). The interaction of Stimulus and Electrode occurred because the Nc amplitude to the novel stimulus decreased from frontal to posterior brain locations.

Figure 6.7: Main effect of Electrode on mean negative component*



*Mean Nc to the novel stimuli for all children

6.5. Discussion

Event related potentials are related to cognitive functions. There is evidence to show that the latencies of the N200 and P3a ERP components decrease with age due to maturation processes.

The main findings of the study showed that children exposed to severe falciparum malaria had significantly longer auditory N200 and P3a latencies than the community controls. The N200 component is associated with a reorienting response to a deviant stimulus and these results suggest that children exposed to severe falciparum malaria had a longer reorientation time compared to community controls. Previous studies have shown that the N200 latency decreases with maturation (Ceponiene, Yaguchi et al., 2002; Fuchigami et al., 1993) with the reduction in N200 correlating highly with age-related improvements in task performance (decreased reaction time and commission errors, sensory processes) (Ceponiene, Yaguchi et al., 2002; Johnstone & Barry, 1996), reflecting maturation of the stimulus discrimination process. The P3a component in children is associated with an involuntary orienting/investigative response (Sokolov et al., 2002) to distracting environmental stimuli. It serves as a probe to frontal lobe functions, especially those associated with orienting of attention to novel events (Escera, Alho, Schroger, & Winkler, 2000; Friedman, Cycowicz, & Gaeta, 2001). In the present study, only children with a history of CM consistently had longer P3a latencies compared to community controls. Previous research on children with a history of severe malaria and/or CM using neuropsychological tests have suggested impairment of attention in these children (Boivin, 2002; Holding et al., 2004).

Novelty oddball paradigms that are methodologically similar with an inter-stimulus interval of less than a second, have been previously administered to school age children in different parts of the world and identified similar ERP components as the present study (Albrecht et al., 2000; Bishop et al., 2007; Ceponiene et al., 1998;

Ceponiene, Yaguchi et al., 2002; Courchesne, 1990; Ponton et al., 2000). This finding was an important step in proposing to use ERPs in a rural setting like Kenya.

In the present study, the longer N200 and P3a latencies among the children with a history of severe falciparum malaria may be suggestive of slower or delayed development in these groups of children especially those with a history of CM compared to community controls. The longer N200 latencies and smaller N2 amplitudes in children with a history of severe falciparum malaria compared with unexposed community controls may suggest a dysfunction in the networks that underlie their production including attention and response selection.

In conclusion, the findings of the present study suggest that while children with a history of severe falciparum malaria have comparable ERP components with community controls, but have an abnormal response to novel stimuli. This was demonstrated by the longer N200 latencies and smaller amplitudes to novel stimuli in all children with a history of severe malaria and prolonged P3a latencies in children with a history of CM. Longer N200 and P3a latencies may be evidence of a maturational delay of the CNS functioning in children with a history of severe falciparum malaria.

CHAPTER 7

The effect of Pneumococcal Meningitis on cognition as measured by Event Related Potentials

7.1 Introduction

The epidemiology of bacterial meningitis in childhood has changed significantly during the past few years, mainly because of programmes for routine immunization of infants with conjugate *Haemophilus influenzae* Type b (Hib) vaccine. Currently, Hib meningitis has declined dramatically and *Streptococcus pneumoniae* and *Neisseria meningitidis* have become the most common agents of bacterial meningitis in childhood (Dawson, Emerson, & Burns, 1999; Schuchat et al., 1997). Despite continuous improvements in antibiotic therapy and intensive treatment, pneumococcal meningitis remains a severe and life-threatening disease. In fact, large trials performed during the past 20 years, involving adult or paediatric cases, report case–fatality rates ranging between 4% and 16% and neurological sequelae in over 30% of the survivors (Arditi et al., 1998; Auburtin et al., 2002; Kastenbauer & Pfister, 2003; Kornelisse et al., 1995). In sub-Saharan Africa over 200,000 children aged below 5 years old die of acute bacterial meningitis annually and a large proportion of those who survive develop neurological sequelae (Peltola, 2001).

The neurological impairments associated with ABM include motor deficits, hearing loss, visual disorders, epilepsy and behavioural problems (Grimwood et al., 2000; Grimwood et al., 1995). The intellectual functions of children post bacterial

meningitis have also been reported as being impaired (Anderson et al., 2004; Anderson et al., 1997; Grimwood et al., 2000; H. G. Taylor et al., 1993; H. G. Taylor et al., 1997). Cognitive deficiencies include learning difficulties, short-term memory deficits and poor academic performance (Bedford et al., 2001; Merkelbach et al., 2000; Schmidt et al., 2006). The poor memory in children with a history of bacterial meningitis may be as a result of hippocampal injury in acute bacterial meningitis (Nau, Soto, & Bruck, 1999). Patients surviving the disease showed hippocampal atrophy, as detected by magnetic resonance imaging (Free, Li, Fish, Shorvon, & Stevens, 1996). Brain damage in the hippocampal structure occurs preferentially in the dentate gyrus and thus probably represents the pathological substrate for cognitive impairment and learning disabilities (Nau et al., 1999).

7.1.1 Pathophysiology of Pneumococcal Meningitis

Bacterial meningitis caused by *Streptococcus pneumoniae* is a severe infectious disease of the CNS and is associated with acute inflammation and subsequent brain damage. In spite of effective antimicrobial therapy and intensive care, the outcome of pneumococcal meningitis remains poor with a mortality rate of up to 30% and permanent sequelae due to neuronal injury in up to 50% of the survivors (Schuchat et al., 1997; van de Beek et al., 2004; Weisfelt, van de Beek, Spanjaard, Reitsma, & de Gans, 2006). Two pathophysiologically different forms of neuronal damage, namely cortical necrosis and hippocampal apoptosis, have been demonstrated in the human disease and corresponding animal models of pneumococcal meningitis (Bifrare, Gianinazzi, Imboden, Leib, & Tauber, 2003; Grandgirard et al., 2007; Nau et al., 1999). Both, the host inflammatory reaction and the pathogen contribute to

the brain damage resulting from pneumococcal meningitis (Grandgirard & Leib, 2006; Koedel, Winkler, Angele, Fontana, & Pfister, 2002).

Methodological limitations in neuropsychological evaluation in Africa may have contributed to the paucity of data on cognitive outcome of children post bacterial meningitis in Africa. Further, the neurological deficits post disease complicates the usefulness of some neuropsychological test. I used event related potentials to detect cognitive impairment in children after pneumococcal meningitis.

7.2. Methods

7.2.1 *Subjects*

Sixty-five children (32 male; 33 female) aged between 4-15 years old were recruited from the Kilifi District Hospital database who had been admitted with pneumococcal meningitis during the period 1994-2004. Their mean age was 8.05 (SD= 3.06) years. An age-matched control group 83 children (39 male; 44 female) with a mean age of 7.89 (SD= 2.85) years was recruited from the DSS maintained in the Kilifi District Hospital.

A comparison between the disease groups and control groups was done to determine whether they differed on the independent measures of age, sex, SES, head-circumference and Schooling. The results show that the groups were similar and there was no statistical significance in any of the measures (Table 7.1).

Table 7.1: Comparison of independent variables between cases and controls

	CT (n= 83) mean± SD	PM (n= 65) mean± SD	F	p-value
OFC* (cm)	50.6 ± 2.3	50.7 ± 2.2	0.014	0.905
Age (yrs)	7.89 ± 2.9	8.05 ± 3.1	0.101	0.752
Sex (Male)	47%	49%	0.073	0.788

*OFC represents the occipito-frontal head-circumference

7.2.2. *Visual and auditory screening*

In children 5 years or older, visual screening and auditory screening was performed using the Sonksen-Silver Visual Acuity Test (Salt et al., 1995) and Kamplex KS16 screening audiometer (P.C. Werth, UK) respectively. The assessment of children below the age of 5 years old was different. Visual acuity assessment was done using the acuity card procedure whereby a coloured object was shown to the child through a small hole in a card, the maximum distance at which the child could fix and follow the object or maintain central, steady fixation was used to determine visual acuity (Hartmann, Ellis, Morgan, Love, & May, 1990). Auditory screening of children aged 5 years old and below involved a distraction test based on the principle that when a sound is presented to a child using distance calibrated warble tone or narrow noises, the child responds by turning the head to locate the source of sound (Egan, 1990).

Children with severe (unable to hear 81 – 105dB on better ear) to profound (over 106 dB) hearing impairment were excluded from the auditory paradigm analyses. Five children (7.7%) exposed to pneumococcal meningitis had severe to profound

hearing loss detected by Kamplex audiometer (PC Werth, UK). None of the control children was detected with severe hearing loss.

7.2.3 *ERP Recording*

In children younger than 5 years old, electrodes were individually positioned at the midline brain locations (i.e. Fz, Fcz, Cz and Pz) using the international 10-20 system (Jasper 1958). In the older children, a greater number of electrodes were used for recording including temporal site (i.e. F7, F8, T3, T4, T5, T6, P3, and P4), frontal (i.e. Fp1 and Fp2), occipital (i.e. O1 and O2) and mastoids (i.e. A1 and A2). All auditory data were collected using a 1000 msec recording epoch with a 200 msec pre-stimulus baseline while the visual data had a 1500 msec recording epoch and a 200 msec baseline. All impedances were less or equal to 8.2 kΩ (band-pass 0.1 to 70 Hz).

A more detailed section of the methods and statistical analysis methods used is given in Chapter 4 of the thesis.

7.3. Results

7.3.1 Auditory ERP results

The P100, N200 and P3a components in the auditory "oddball" paradigm were analysed. The P100 component was defined as the highest peak between 60 and 130 msec post stimulus presentation while the N200 component was defined as the most negative point between 120 and 220 msec. The P3a component was defined as the most positive point occurring between 250 and 450 msec. Amplitudes and

latencies for these components have been analysed and their results summarised below.

The amplitudes and latencies of each of the ERP components are shown in Table 7.2. The latencies of auditory N200 to the novel stimulus for the children with a history of pneumococcal meningitis were significantly longer at Fcz, Cz and Pz (Table 7.2a). Children with a history of pneumococcal meningitis had shorter P100 and N200 amplitudes but the differences did not reach significance (Table 7.2b).

Table 7.2a: Comparison of mean latencies of pneumococcal meningitis and controls to auditory novel stimulus

Latency		Pneumococcal Meningitis		Control	
		Mean (msec)	SD	Mean (msec)	SD
P100	**Fz**	89.4	18.1	90.2	18.4
	Fcz	89.7	17.8	88.3	17.6
	Cz	88.3	16.8	86.7	18.0
	Pz	86.2	15.3	85.8	23.2
N200	**Fz**	**228.6***	35.0	211.6	47.3
	Fcz	219.1	36.4	205.2	51.7
	Cz	**226.6****	38.1	203.2	55.9
	Pz	**231.9****	31.6	212.8	46.5
P3a	**Fz**	322.7	38.3	324.6	38.8
	Fcz	309.8	34.7	314.9	36.1
	Cz	309.0	39.7	310.3	37.2
	Pz	333.3	34.8	336.2	39.4

The difference was determined by means of independent samples 2-tailed t-test: ***P< 0.05 **P< 0.01**

Table 7.2b: Comparison of mean amplitude of pneumococcal meningitis and controls to auditory novel stimulus

Amplitude		Pneumococcal Meningitis		Control	
		Mean (μV)	SD	Mean (μV)	SD
P100	**Fz**	6.6	5.7	7.9	5.2
	Fcz	6.9	6.3	8.0	5.2
	Cz	5.4	5.4	7.0	4.8
	Pz	1.7	4.2	3.0	3.9
N200	**Fz**	-0.7	9.5	-2.6	7.7
	Fcz	3.1	9.2	1.1	7.5
	Cz	1.6	9.2	1.2	6.9
	Pz	-3.4	7.7	-5.1	6.0
P3a	**Fz**	9.7	10.5	7.8	7.1
	Fcz	13.7	11.0	12.0	7.7
	Cz	10.8	10.2	10.0	7.5
	Pz	6.8	9.3	6.4	6.6

The difference was determined by means of independent samples 2-tailed t-test: ***P< 0.05 **P< 0.01**

7.3.1.1 *AUDITORY P100*

Latency: The P100 latency had significant main effects of Stimulus, F (2, 208) = 5.517 (p= 0.005) and Electrode, F (3,312) = 3.432 (p = 0.038). *Post hoc* analyses revealed a trend towards shorter P100 latencies to the infrequent and novel stimuli but no trend was evident in electrode site. There were also significant interactions of Stimulus by Electrode (F (6, 624) = 2.899, p= 0.022) and Diagnosis by Age (F (9, 104) = 2.361, p= 0.018). The interaction of Diagnosis by Age occurred because children with exposure to pneumococcal meningitis had increasing P100 latencies to novel stimuli with increasing age, while unexposed age-matched controls had the latencies decreasing with age. The interaction of stimulus by electrode was driven by latencies of the auditory P100 component being shorter for novel and infrequent stimuli than for frequent stimulus at fronto-central electrodes.

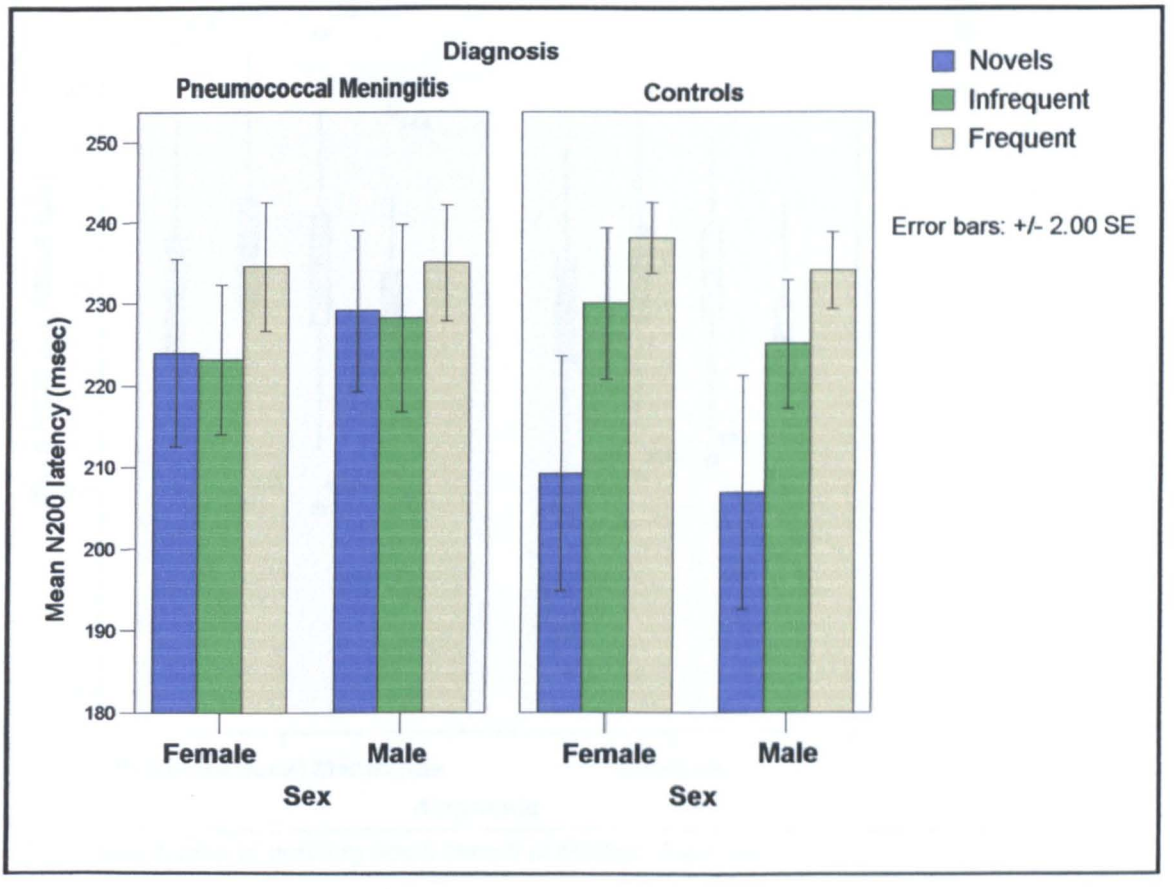
Amplitude: In the amplitude for the P100 component was largest for the novel stimulus (main effect of Stimulus F (2, 208) = 9.162, p< 0.001). Repeated measures revealed that P100 amplitude was significantly greater at both Fz and Fcz compared to Pz (main effect of Electrode F (3, 312) = 98.097, p< 0.001). The P100 amplitude decreased with increasing age F (10, 104) = 2.223 (p= 0.022). There were also interaction effects Stimulus by Electrode F (6, 624) = 6.848 (p< 0.001) due to larger amplitudes for the novel stimulus at Fz, Fcz and Cz brain sites. There was a significant interaction of electrode by Diagnosis by Age F (27, 312) = 1.888 (p= 0.035). This occurred due differential changes in the P100 amplitude by age and diagnosis at Fz and Fcz; while the P100 amplitude of children exposed to pneumococcal meningitis decreased with increasing age from 4-15 years, those of the unexposed children first increased between 4-7 years and then decreased from 8-15 years. When the amplitude of the auditory P100 to the frequent stimuli was compared for cases without hearing impairment and community controls, children with exposure to pneumococcal meningitis had significantly smaller amplitude in fronto-central (Fcz) electrodes.

7.3.1.2 *AUDITORY N200*

Latency: The peak latency of the N200 component for the novel stimuli was significantly shorter than that of that the frequent stimuli (main effect of Stimulus F (2, 208) = 16.189, p< 0.001). There is decrease in the N200 latency for the novel stimulus from age 4-9 years for unexposed children but the latency increases for those with a history of pneumococcal meningitis and hence an interaction effect of Stimulus by Diagnosis by Age F (18, 208) = 4.318 (p< 0.001). The N200 latency to

the novel stimulus for females was shorter than that of the frequent stimuli for both exposed and unexposed children but the same was not true for exposed males, Diagnosis by Sex F (1, 104) = 8.026 (p= 0.006) (Figure 7.1).

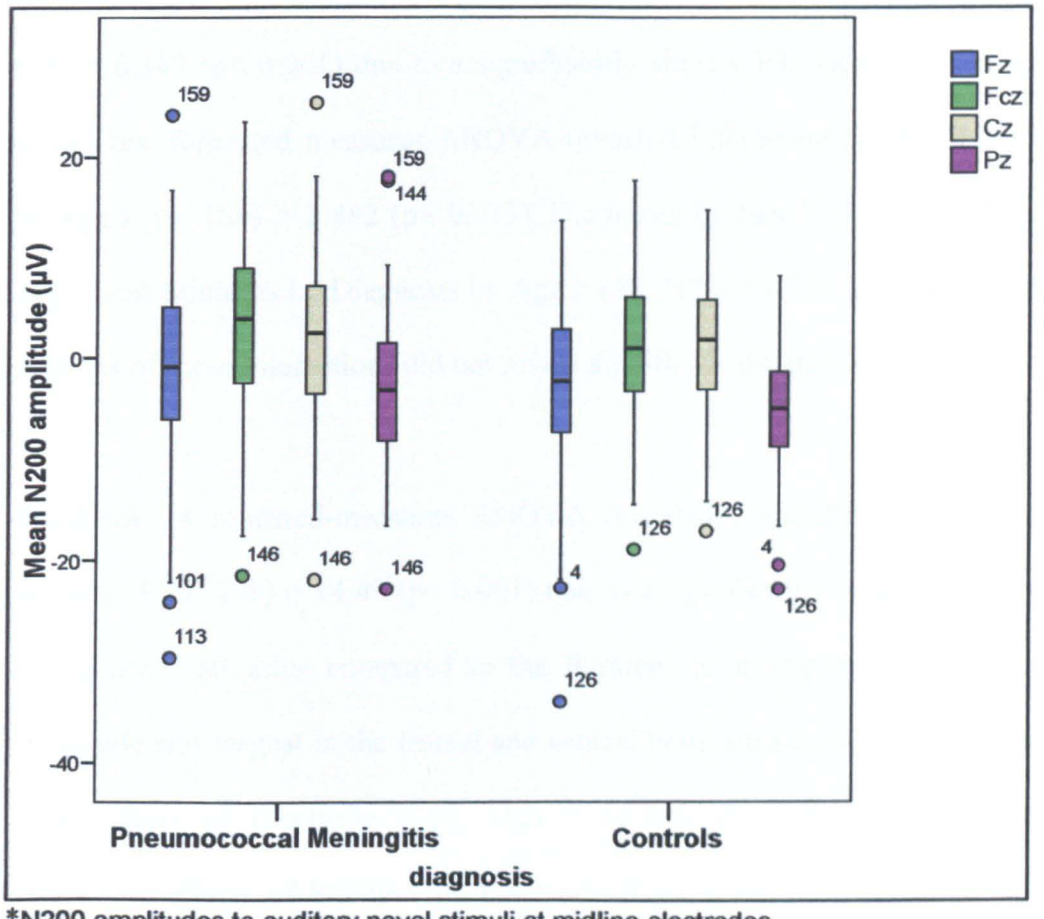
Figure 7.1: N200 latency for males and females by diagnosis



Amplitude: The N200 amplitude to the novel stimulus was significantly smaller than that of the frequent or infrequent stimuli giving a main effect of Stimulus F (2, 208) = 41.54 (p< 0.001). The N200 peak was greatest in the frontal brain electrodes and decrease posteriorly giving a main effect of Electrode F (3, 312) = 16.13 (p< 0.001). Children who were exposed to pneumococcal meningitis had borderline significance smaller N200 amplitudes (main effect of Diagnosis F (1, 104) = 7.948, p= 0.006) at all electrodes (interaction effect of Electrode by Diagnosis F (3, 312) = 3.205, p= 0.049). This result was possibly due to outliers that could have biased the

mean (Figure 7.2). The amplitude of N200 decreased with increasing age (main effect of Age, F (10, 104) = 2.155, p= 0.026).

Figure 7.2: Boxplot of N200 amplitudes by diagnosis*



*N200 amplitudes to auditory novel stimuli at midline electrodes

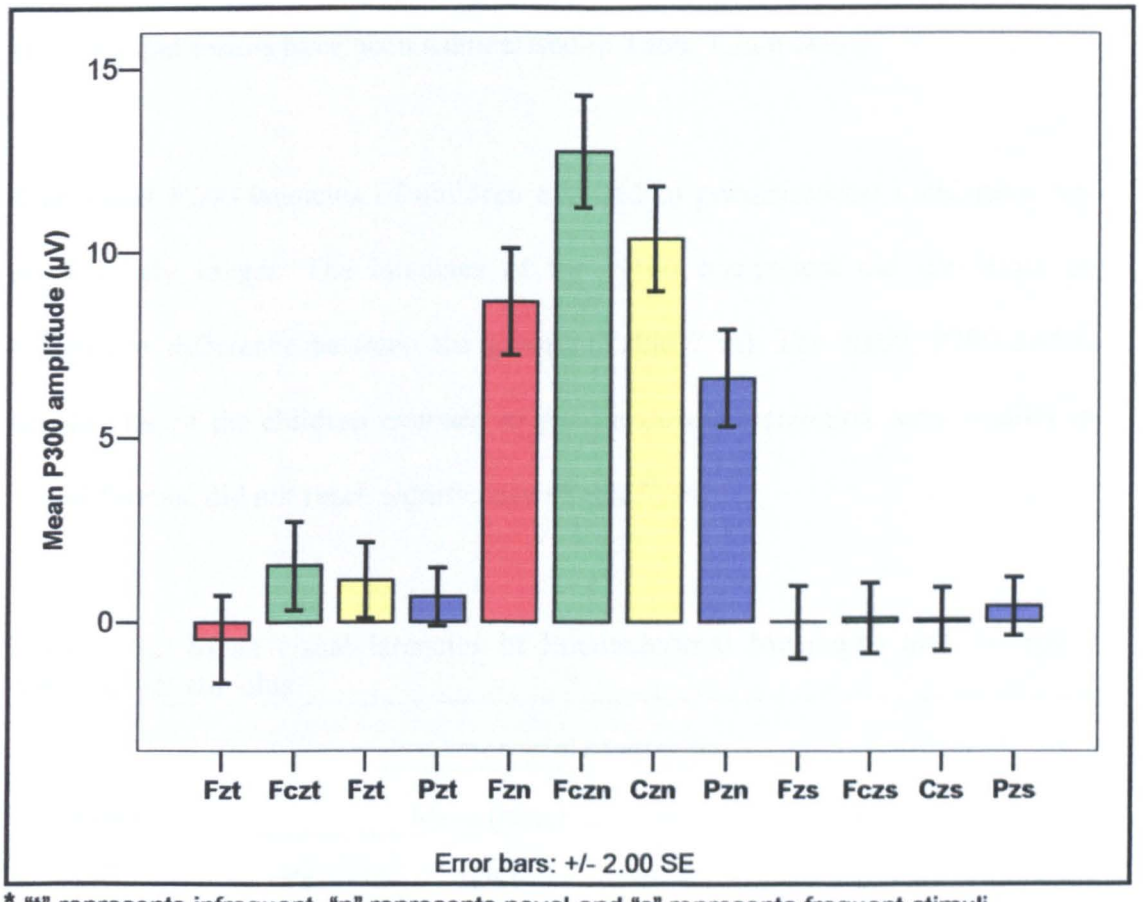
There were also significant interaction effect of Stimulus by Electrode F (6, 624) = 38.06 (p< 0.001). This effect was due to significant N200 differences between stimulus and electrode at Fz, Fcz and Cz; the N200 amplitude to the frequent and infrequent stimuli was significantly longer than that to the novel stimuli at these electrodes.

7.3.1.3 *AUDITORY P3a*

Latency: The P3a latency was shorter at the fronto-central brain electrodes compared to the posterior electrodes (significant main effect of Electrode F (3, 312) = 7.677, p= 0.001). There was an interaction effect of Stimulus by Electrode F (6, 624) = 6.347 (p< 0.001) due to a significantly shorter P3a latency at Fcz and Cz brain sites. Repeated measures ANOVA revealed interaction effects of Diagnosis by Age F (9, 104) = 2.482 (p= 0.013), Diagnosis by Sex F (1, 104) = 4.567 (p= 0.035) and Stimulus by Diagnosis by Age F (18, 208) = 1.734 (p= 0.036). *Post hoc* analyses of these interactions did not reveal significant differences.

Amplitude: A repeated-measures ANOVA revealed a significant main effect of Stimulus F (2, 208) = 94.49 (p< 0.001) due to a significantly larger P3a amplitude to the novel stimulus compared to the frequent or infrequent stimuli. The P3a amplitude was largest in the frontal and central brain sites compared to the parietal (main effect of Electrode F (3, 312) = 16.898, p= 0.001). There were also interaction effects of Stimulus by Electrode (F (6, 624) = 23.436, p< 0.001) that occurred due to significantly larger P3a amplitude to the novel and infrequent stimulus than frequent stimuli at Fcz and Cz brain sites (Figure 7.3).

Figure 7.3: P3a amplitude: Stimulus by electrode



* "t" represents infrequent, "n" represents novel and "s" represents frequent stimuli.

There was a significant interaction effect of Electrode by Diagnosis ($F(3, 312) = 3.954$, $p = 0.026$) but *post hoc* analysis did not show significant differences in the amplitudes of exposed and unexposed children. The P3a amplitude of children exposed to pneumococcal meningitis remained constant throughout the period of 4-15 years but that of unexposed children increased with increasing age (Diagnosis by Age $F(9, 104) = 2.668$, $p = 0.008$).

7.3.2 Visual ERP results

The visual N100 was defined as the most negative peak between 100-200 msec, while P200 (P250) was defined as the most positive peak between 200-300 msec. The negative component, Nc, was defined as the average amplitude between 300

and 850 msec. Mean amplitudes and latencies for the defined components were analysed and results have been summarised in Table 7.2a/b below.

The visual P200 latencies of children exposed to pneumococcal meningitis were significantly longer. The latencies of the N100 component did not show any significant difference between the groups (Table 7.3a). The N100, P200 and Nc amplitudes of the children exposed to pneumococcal meningitis were smaller but the difference did not reach significance (Table 7.3b).

Table 7.3a: Mean visual latencies of Pneumococcal Meningitis and controls to visual novel stimulus

		Pneumococcal Meningitis		Control	
Latency		Mean (msec)	SD	Mean (msec)	SD
N100	vFz	156.6	29.8	153.2	25.0
	vFcz	156.2	31.0	153.0	27.2
	vCz	156.3	32.1	155.1	30.4
	vPz	173.5	39.2	177.8	43.6
P200	vFz	268.2	44.1	257.3	38.0
	vFcz	**276.8****	47.3	257.1	39.5
	vCz	**280.4****	48.2	255.7	40.8
	vPz	**304.0***	50.2	287.0	51.2

The difference was determined by means of independent samples 2-tailed t-test: ***P< 0.05 **P< 0.01**

Table 7.3b: Mean visual amplitudes of Pneumococcal Meningitis and controls to novel stimulus

		Pneumococcal meningitis		Control	
Amplitude		Mean (μV)	SD	Mean (μV)	SD
N100	vFz	-10.4	7.3	-11.2	6.2
	vFcz	-10.1	6.9	-11.0	5.9
	vCz	-10.3	6.6	-10.9	6.0
	vPz	-7.3	7.6	-7.5	6.9
P200	vFz	2.0	9.8	1.5	7.2
	vFcz	2.7	10.4	1.7	6.8
	vCz	1.9	9.2	1.2	6.3
	vPz	3.3	8.3	4.0	9.3
Nc	vFz	-6.7	8.9	-7.9	6.8
	vFcz	-4.6	9.5	-4.7	6.2
	vCz	-2.7	8.3	-1.6	6.4
	vPz	4.0	8.2	6.0	8.0

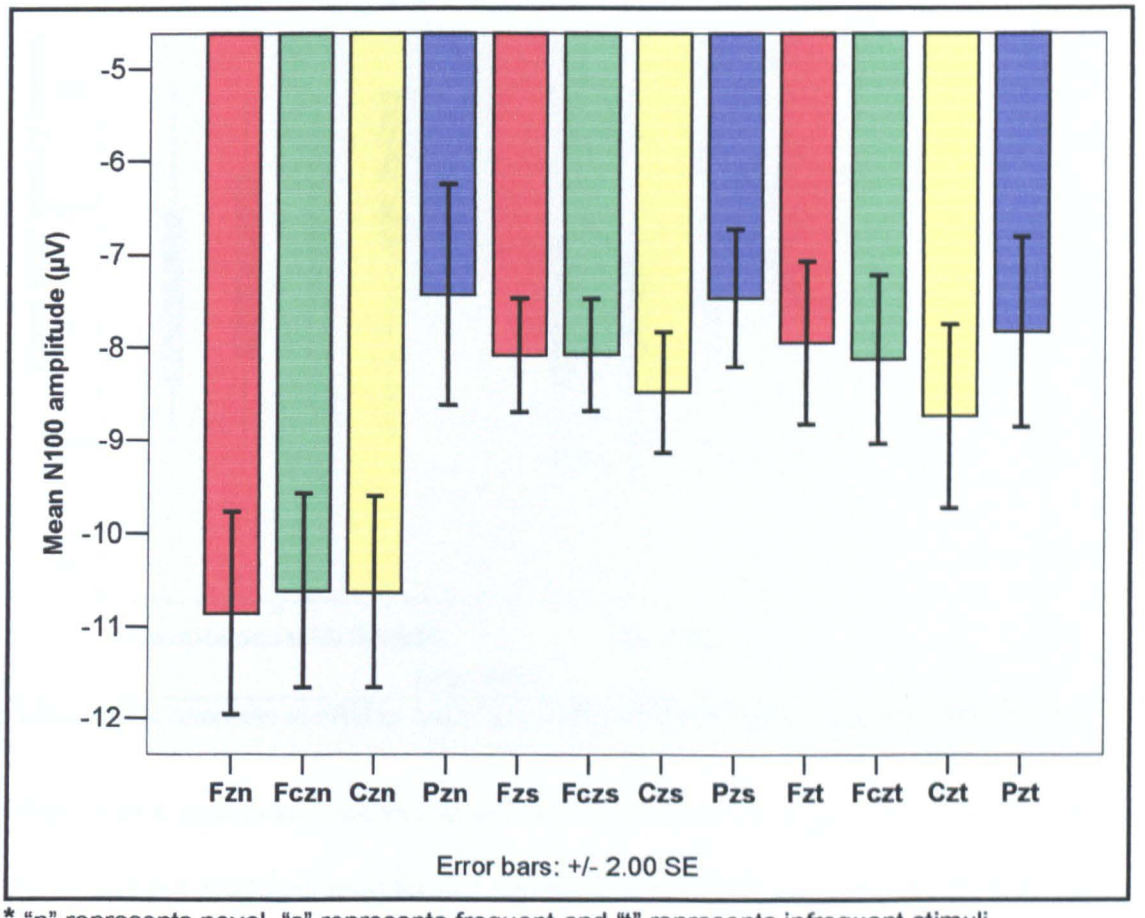
The difference was determined by means of independent samples 2-tailed t-test: ***P< 0.05 **P< 0.01**

7.3.2.1 *VISUAL N100*

Latency: The analysis of the N100 latency using repeated-measures ANOVA revealed a significant main effect of Electrode ($F(3, 315) = 23.324$, $p < 0.001$) due to a longer latency at the posterior brain sites compared to fronto-central electrodes. The N100 latency decreases with increasing age (main effect of Age $F(10, 105) = 2.412$, $p = 0.013$). There was an interaction effect of Electrode by Diagnosis $F(3, 315) = 5.287$ ($p = 0.011$) due to longer N100 latencies for children exposed to pneumococcal meningitis at the frontal and central electrodes. The interaction effect of Age by Sex ($F(9, 105) = 2.231$, $p = 0.025$) occurred because the females had shorter latencies than males of their age and their latencies decreased with increasing age.

Amplitude: There was a trend in the amplitude of the N100 component being larger for the novel stimulus than the other stimuli (F (2, 210) = 3.320, p= 0.047). There was a significant interaction effect of Stimulus by Electrode (F (6, 630) = 4.294, p= 0.007) due to larger N100 amplitude to the novel stimulus in the fronto-central electrodes (Fzn, Fczn, Czn) than other stimuli (Figure 7.4).

Figure 7.4: N100 amplitude: electrode by stimulus



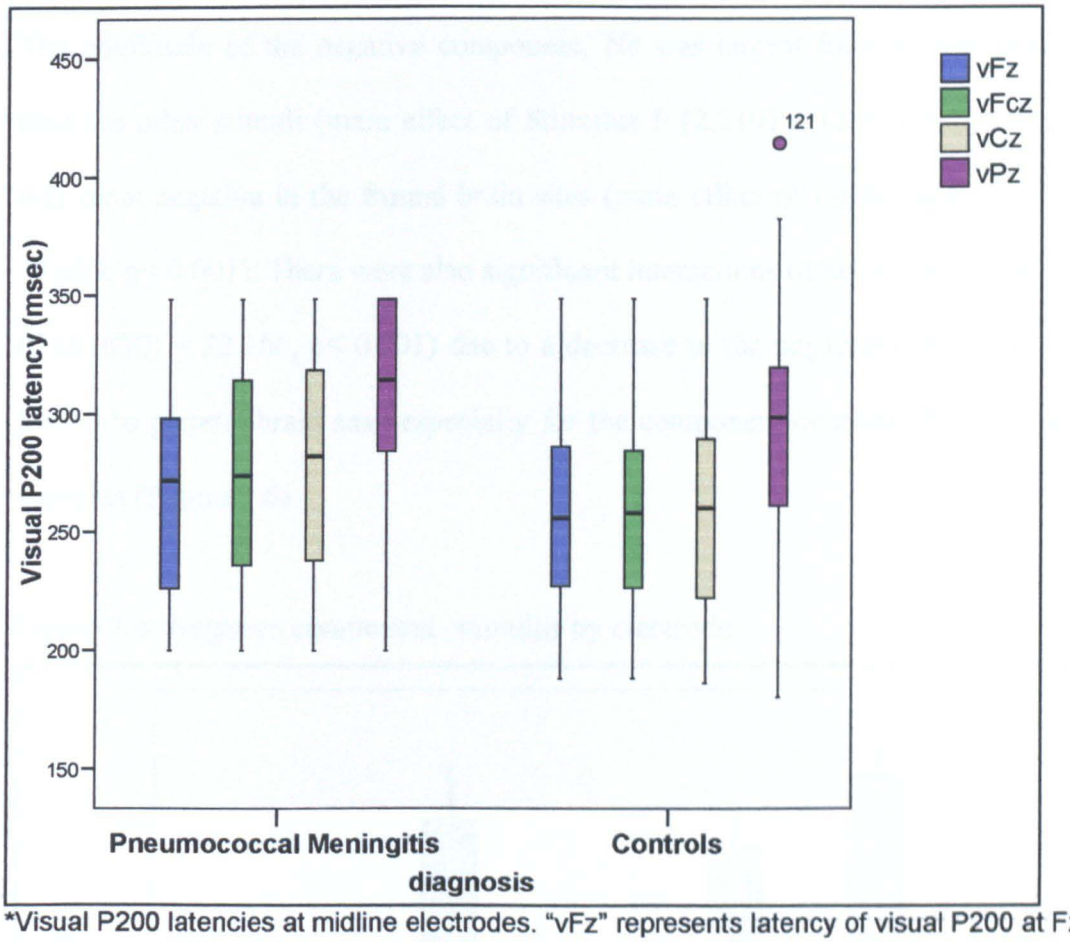
* "n" represents novel, "s" represents frequent and "t" represents infrequent stimuli.

7.3.2.2 *VISUAL P200*

Latency: A repeated-measures ANOVA of the latencies of P200 reveals a significant effect of electrode due to a significantly longer latency at Pz (F (3, 315) = 18.502, p< 0.001). There was a significant main effect of Diagnosis (F (1, 105) =

15.27, p< 0.001) due to longer P200 latencies for children exposed to pneumococcal meningitis compared to controls (Figure 7.5).

Figure 7.5: Boxplot showing visual P200 latencies by diagnosis*



*Visual P200 latencies at midline electrodes. "vFz" represents latency of visual P200 at Fz

There was a significant interaction effect of Stimulus by Age (F (20, 210) = 1.953, p= 0.013) but *post hoc* analysis did not show differences between the stimulus by age.

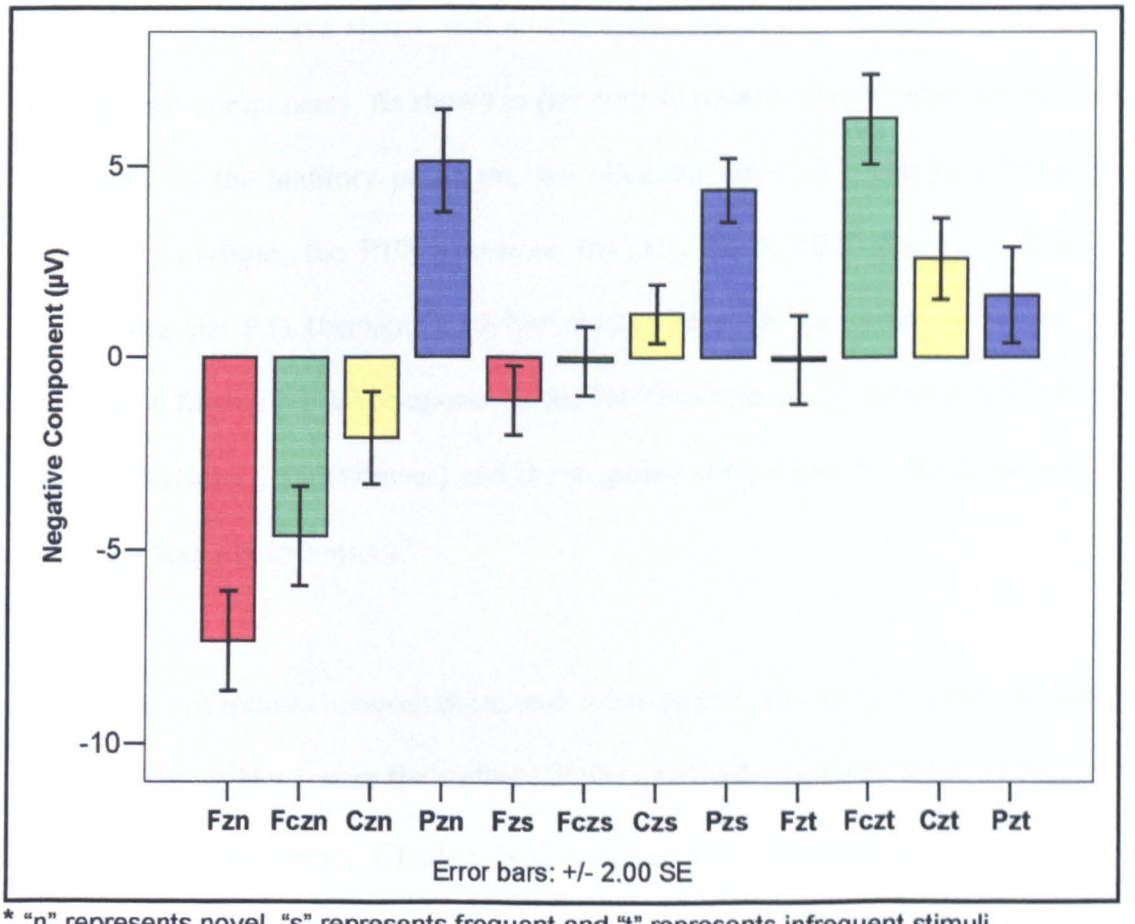
Amplitude: The P200 amplitude to the novel stimulus was significantly shorter than that of the frequent or infrequent (main effect of Stimulus F (2, 210) = 12.871, p< 0.001). There was a significant interaction of Electrode by Sex (F (3, 315) = 3.657,

p= 0.040) due to larger P200 amplitudes for females than males in the posterior electrodes.

7.3.2.3 *NEGATIVE COMPONENT, Nc*

The amplitude of the negative component, Nc was largest for the novel stimulus than the other stimuli (main effect of Stimulus F (2,210) = 12.978, p< 0.001) and was most negative in the frontal brain sites (main effect of Electrode F (3, 315) = 37.689, p< 0.001). There were also significant interactions of Stimulus by Electrode (F (6, 630) = 32.166, p< 0.001) due to a decrease in the negative component from frontal to parietal brain sites especially for the component obtained from the novel stimulus (Figure 7.6).

Figure 7.6: Negative component: stimulus by electrode



* "n" represents novel, "s" represents frequent and "t" represents infrequent stimuli.

Repeated measures ANOVA had revealed interaction effects of Electrode by Sex ($F(3, 315) = 8.171$, $p < 0.001$), Diagnosis by Age by Sex ($F(6, 105) = 2.904$, $p = 0.012$) and Stimulus by Electrode by Sex ($F(6, 630) = 5.599$, $p = 0.003$) but *post hoc* analyses did not reveal any significant differences.

7.4. Discussion

The aim of the present study was two-fold; the first was to provide evidence of the novelty orienting response in both the auditory and visual modalities and second was to distinguish between children with a history of pneumococcal meningitis and those unexposed to the disease.

The study results have shown that all children, exposed or unexposed, exhibited typical ERP components. As shown in previous literature using an inter-stimulus of 700 msec in the auditory paradigm, we obtained three ERP components in the auditory paradigm; the P100 (between 60-130 msec), N200 (between 120-220 msec) and the P3a (between 250-450 msec). Also, in the visual paradigm, we obtained a further 3 ERP components; the N100 (between 100-200 msec), P200 (or P250) (between 200-300 msec) and the negative component, Nc (mean amplitude between 300 and 850 msec).

The main differences between those who were exposed to pneumococcal meningitis and normal children were the auditory P100 amplitude, auditory N200 latency and the visual P200 latency. Children with a history of pneumococcal meningitis had decreased auditory P100 amplitudes, increased auditory N200 latencies and

increased visual P200 latencies compared to their age-matched controls. The auditory P100 amplitude increases with age (Ceponiene, Rinne, & Naatanen, 2002; Ponton et al., 2000) and may be used as an indicator of maturation. The results may suggest that children with a history of pneumococcal meningitis may have delayed maturation compared to their age-matched controls. The P100 component is thought to be an indicator of preferential attention to sensory inputs (Key et al., 2005) and is thought to be localized at the superior temporal gyrus (Thoma et al, 2003). The superior temporal gyrus contains several important structures of the brain, including the Brodmann's area (sensation of sound) and the Wernicke's area (processing of speech). Previous studies found that children with a history of bacterial meningitis were at risk for language difficulties post illness (Anderson et al., 1997; Pentland, Anderson, & Wrennall, 2000). The results suggest that bacterial meningitis may result in a delay in language development as suggested by our result of longer P100 component in these children. The auditory N200 latency is influenced by attention and task difficulty (Naatanen, 1992). The results of the present study showing increased auditory N200 latency suggest that pneumococcal meningitis may impair the children's attention. The N200 component is said to result from a deviation in form or context of a prevailing stimuli (Naatanen & Picton, 1986). In the present study, novel sounds (environmental noises) provided a deviation from the frequent (1000Hz SPL) and infrequent tones (2000Hz SPL) that acted as the prevailing stimuli. The visual P200 may index mechanisms of selective attention (Hackley, Woldorff, & Hillyard, 1990), feature detection processes (Luck & Hillyard, 1994), and other early sensory stages of item encoding. The prolonged visual P200 latencies in children with a history of pneumococcal meningitis relative

to those of unexposed peers may suggest impaired attention and poorer detection processes.

In the present study, children with profound to severe hearing loss were excluded from the ERP paradigms to minimize biases arising from sensory impairments. However, it is possible that subtle hearing loss and cortical blindness could have accounted for the differences in children with pneumococcal meningitis. The auditory N200 and visual P200 latencies are indices of processing speed and attention allocation and could have been affected by these subtle impairments.

Previous research has reported potentially serious cognitive consequences in children with a history of pneumococcal meningitis (Grimwood et al., 2000; Schmidt et al., 2006). These children have lower IQ's and performed poorer on neuropsychological tests than their age mates many years post infection. This may have negative effects on their academic outcomes and cognitive development in general.

CHAPTER 8

ERPs and HIV encephalopathy

8.1. Introduction

Each year, more than 530,000 young children (90 percent of whom live in sub-Saharan Africa) become infected with HIV; most (about 95 percent) of these children become infected *in utero* (before birth), during delivery or through breast milk (Coovadia et al., 2007; UNAIDS, 2007). The effects of HIV infection on children's psychological growth and development can range from mild to devastating (Smith et al, 2006). Studies have shown delays in mental and motor development of very young children with vertically transmitted HIV infection (Chase et al., 2000; Wolters, Brouwers, Moss, & Pizzo, 1995). HIV infection is known to produce severe cognitive and neurological impairments in many AIDS patients (Bungener, Le Houezec, Pierson, & Jouvent, 1996). Advances in medical treatment of children with HIV have helped prolong survival and quality of life. However with the anticipated decrease in childhood mortality, the impact of HIV-associated disability will become increasingly important to practitioners and policy makers (WHO, 2005). The present study seeks to compare the auditory and visual event related potentials (ERPs) of children who were HIV infected to those of age-matched unexposed children with a view of determining whether ERPs can detect cognitive impairment due to HIV infection and determine the pattern of this impairment.

8.1.1 Pathophysiology of HIV

The human immunodeficiency virus infects lymphocytes, gains access to the brain parenchyma, with infection of the microglia and to a lesser extent astroglia. It is hypothesized that HIV infection leads to neurotoxicity through secretions by these infected cells, neuronal damage and disturbances in the communication between cells (Angelini et al., 2000). The white matter appears to be particularly infected. Post-mortem studies of children with progressive HIV encephalopathy have indicated cerebral atrophy and ventricular enlargements, calcifications in the basal ganglia and cerebellum and a reduction in white matter and demyelination (Angelini et al., 2000; Safriel, Haller, Lefton, & Obedian, 2000).

8.2. Methods

8.2.1. *Subjects*

Forty children (18 male; 22 female) aged between 18-40 months were recruited from the Kilifi District Hospital's Comprehensive Care Clinic for HIV affected and infected patients and families. These are children born to HIV positive mothers and attend regular clinics at the centre as they benefit from free drugs from the KDH. These children did not have clinical signs of infection. These were compared with an age-matched group of 39 sero-negative children (21 male; 18 female) living in the same community and recruited from siblings of children who came to the Care clinic. These children had been tested and were known to be sero-negative. The independent variables, age, sex and head-circumference of the HIV and control groups were compared. One-way ANOVA showed that they were similar on age and sex but there was borderline difference in the occipito-frontal circumference

with HIV+ children having smaller head (Table 8.1). The group status of the children remained unknown to all ERP technicians during the data collection stage.

Table 8.1: Comparison of independent variables of HIV and control groups

	CT (n= 39) mean± SD	HIV+ (n= 40) mean± SD	F	p-value
OFC* (cm)	47.66 ± 1.7	46.90 ± 1.7	3.496	0.065
Age (months)	27.1 ± 4.7	27.3 ± 5.6	0.023	0.881
Sex (% Male)	54%	45%	0.607	0.438

*OFC represents the occipito-frontal head-circumference

In the auditory paradigm, a three-stimulus novelty oddball paradigm was used in which a series of pure tones and novel sounds was presented to all the children. This consisted of: (i) frequently presented tone (1000 Hz, 200 msec long, 5msec rise and fall time, 75 dB SPL, 80% probability); (ii) infrequent tone (2000 Hz, 200 msec long, 5 msec rise and fall time, 75 dB SPL, 10% probability); (iii) environmental sounds "novel noises". In the visual experiment, a three-stimulus paradigm was also used. It consisted of: (i) frequently presented face (80% probability); (ii) infrequently presented face (10% probability); (iii) novel "abstract" art (10% probability). The children were normally seated on their mother's lap facing the computer monitor and were awake during the duration of the experiment. On average, the auditory task took 8 minutes and the visual 10 minutes to complete.

8.2.2. ERP recording

The electrodes were individually positioned at the midline brain locations (i.e. Fz, Cz and Pz) using the international 10-20 system (Jasper, 1958). Continuous EEG

data were referenced to Cz and then re-referenced offline to averaged mastoids. The ground electrode was placed on the forehead (Fpz). Eye-blinks were recorded from bi-polar channels attached separately above and below the eye. All auditory data were collected using a 1000 msec recording epoch with a 200 msec pre-stimulus baseline while the visual data had a 1500 msec recording epoch and a 200 msec baseline. All impedances were less or equal to 8.2 kΩ (band-pass 0.1 to 70 Hz).

Electroencephalographic and EOG channels were recorded using SCAN (version 4.3, Neuroscan®, Compumedics, El Paso, Texas, USA) acquisition system and amplified with Nu-amps amplifier. Continuous EEG data were recorded at a sampling rate of 500 Hz, low pass filtered offline at 20Hz. The resulting epochs were baseline corrected and excluded if they exceeded 150μV in either direction.

Two components were recorded in the auditory paradigm i.e the P100 and the P200 and a further three i.e the N100, P200 and negative component, Nc.

8.2.3 Statistical analysis

In the analysis of the ERP components using repeated measures analysis of variance, we included age and sex into the model. Age was grouped into three bands; 18-24 months, 25-29 months and 30-40 months. The Greenhouse-Geisser correction was used to correct for sphericity where applicable. Level of significance was set at $p < 0.05$.

8.3. Results

The mean age of the 40 children infected with HIV was 27.2 months (SD= 5.6 months). The age-matched control group had 39 children (21 male; 18 female) with a mean age of 27.1 months (SD= 4.7 months). The results of two children (both HIV infected) were omitted from the final analysis due to excessive artefact. The children were grouped according to their age as shown in table 8.2.

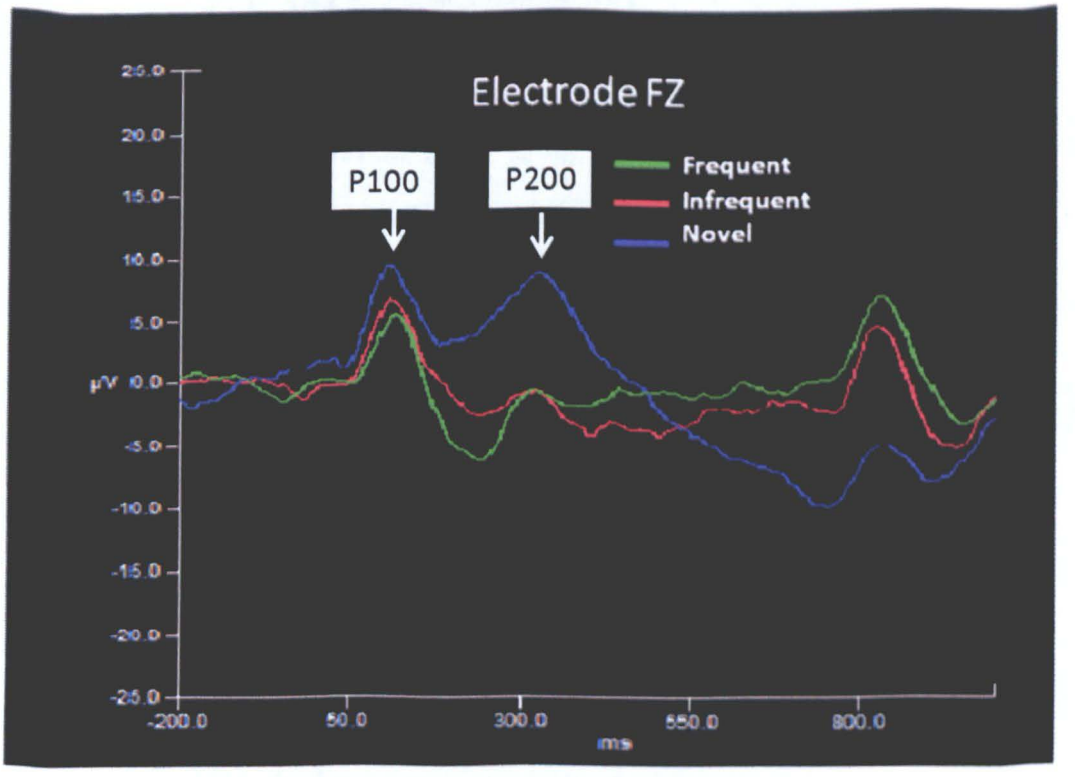
Table 8.2: Age by diagnosis of the study sample

	AGE			
	18 – 24 months	**25 – 29 months**	**30 – 40 months**	**Total**
HIV infected	8	20	12	**40**
Study controls	8	21	10	**39**
Total	**16**	**41**	**22**	**79**

8.3.1. Auditory ERP results

The P100 and P200 components in the auditory "oddball" paradigm were analysed. The P100 component was defined as the highest peak between 100 and 200 msec post stimulus presentation while the P200 component was defined as the most positive point between 200 and 400 msec (Figure 8.1).

Figure 8.1: Grouped averages for controls at Fz



Mean amplitudes and latencies for these components were analyzed and their results have been summarised in table 8.3 below. In the auditory paradigm, the latency of the P100 component was significantly longer for children age 30-40 months exposed to HIV than for unexposed controls.

Table 8.3: Mean auditory components by age and diagnosis in the HIV study

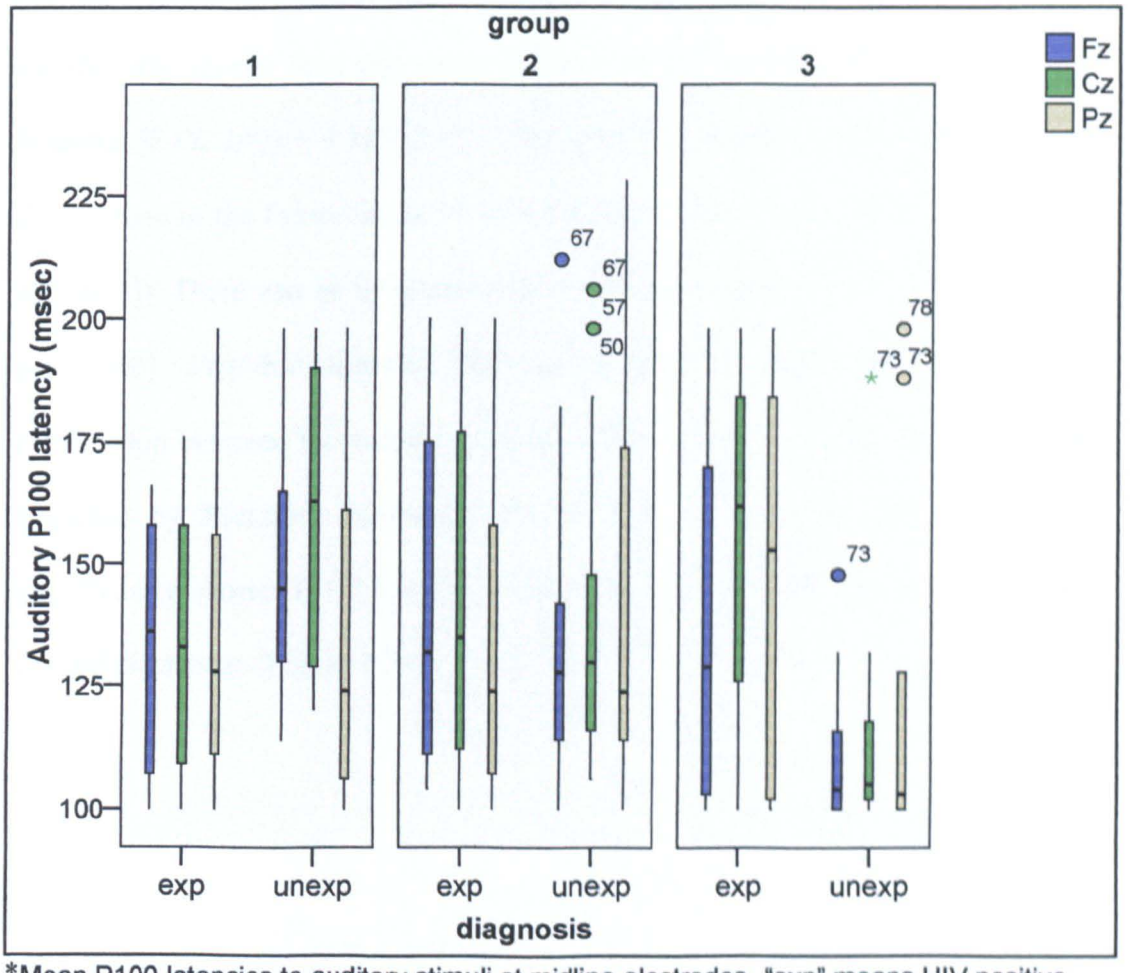
Latency (msec)		18 - 24 months				25 - 29 months				30 - 40 months			
		HIV infected		Controls		HIV infected		Controls		HIV infected		Controls	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
P100	Fz	138.3	26.7	149	26.7	139.9	31.4	132.5	27.3	**136.7***	35.4	111.4	16.4
	Cz	139.4	26.6	160.3	32.4	142.8	34.6	139.5	31.5	**157****	32.8	116.2	27.1
	Pz	141.1	32.4	135	37.7	136.5	33.1	141.7	39.2	147	39.6	123.8	37.6
P200	Fz	284	56.5	276	43.5	294.8	54.2	294.1	56.7	275.7	57.4	291.2	57.7
	Cz	256.3	51.4	233.8	26.2	276.3	53.2	286.5	56	286.8	58.2	281.6	55.4
	Pz	247.1	62	252.5	72.9	263.2	58.9	283.1	60.2	307.7	66.6	278.2	74.2

Amplitude (μV)		18 - 24 months				25 - 29 months				30 - 40 months			
		HIV infected		Controls		HIV infected		Controls		HIV infected		Controls	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
P100	Fz	15.9	9.9	15.3	7.3	13.3	6.3	11.7	9.6	9.5	5.4	12.3	6.5
	Cz	13.8	9.4	15.8	5.5	12.9	6.8	11	6.6	11.6	7.9	11.7	6.3
	Pz	5.7	12.7	8.6	3	7.3	6.5	4.9	6.7	4.6	6.6	3.6	5.3
P200	Fz	13.9	10.2	16.8	10.1	11.9	8.3	6.4	11.6	9.6	8.8	12.5	11.2
	Cz	11.5	7.4	15.9	4.3	10	10.6	5.2	18.2	11.5	8.1	12.2	11.3
	Pz	5.9	5.6	7.4	3.2	1.8	8.5	-0.5	13.2	2.2	9.5	2.8	8.6

8.3.1.1 *AUDITORY P100*

Latency: A repeated-measures ANOVA revealed a main effect of Diagnosis [F (1, 71) = 6.737, p= 0.011], which was influenced by significantly longer latencies of the P100 latency for HIV infected children compared with the controls, (Figure 8.2). There was a significant interaction of Stimulus and Electrode [F (4, 284) = 4.584, p= 0.003] that occurred because the P100 latencies of the infrequent stimuli were shortest at frontal electrode sites and increased posteriorly.

Figure 8.2: Boxplot of diagnosis on P100 latency panelled by age-group*



*Mean P100 latencies to auditory stimuli at midline electrodes. "exp" means HIV positive and "unexp" are controls. Mean P100 latencies averaged for all stimuli. Group 1 is age 18-24 months, group 2 is 25-29 months and group 3 is 30-40 months.

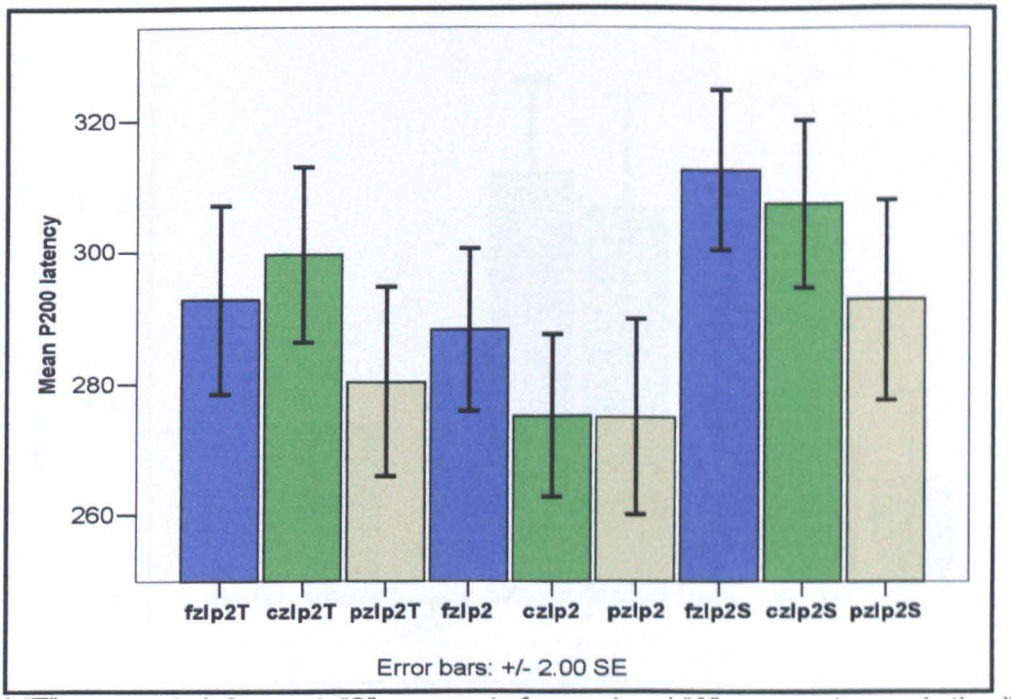
Amplitude: The P100 amplitude for the novel stimulus was the larger than that of the frequent and infrequent stimuli (main effect of Stimulus [F (2, 142) = 9.266, p<

0.001]). The amplitude for the P100 component was smallest at the posterior brain site (Pz). Repeated measures revealed that P100 amplitude was significantly greater at both Fz and Fcz compared to Pz (main effect of Electrode [F (2, 142) = 54.02, p< 0.001]). There were also interaction effects of Stimulus by Electrode [F (4, 284) = 3.152, p= 0.030] due to larger amplitudes for the novel stimulus than other stimuli at Fz and Cz brain sites.

8.3.1.2 *AUDITORY P200*

Latency: The peak latency of the P200 component for the novel stimuli was significantly shorter than that of the frequent or infrequent stimuli (main effect of Stimulus [F (2, 142) = 4.155, p= 0.018]). The P200 latency at Pz was significantly shorter than in the frontal brain sites (main effect of Electrode [F (2, 142) = 6.399, p= 0.004]). There was an interaction effect of Stimulus by Age [F (4, 142) = 3.586, p= 0.008]. *Post-hoc* analyses did not reveal any significant trends in the relationship between the stimulus and age of the children. There was a significant Stimulus by Electrode interaction [F (4, 284) = 2.732, p= 0.039] due to a significantly shorter P200 latency to the novel than frequent stimuli at the fronto-central electrodes (Figure 8.3).

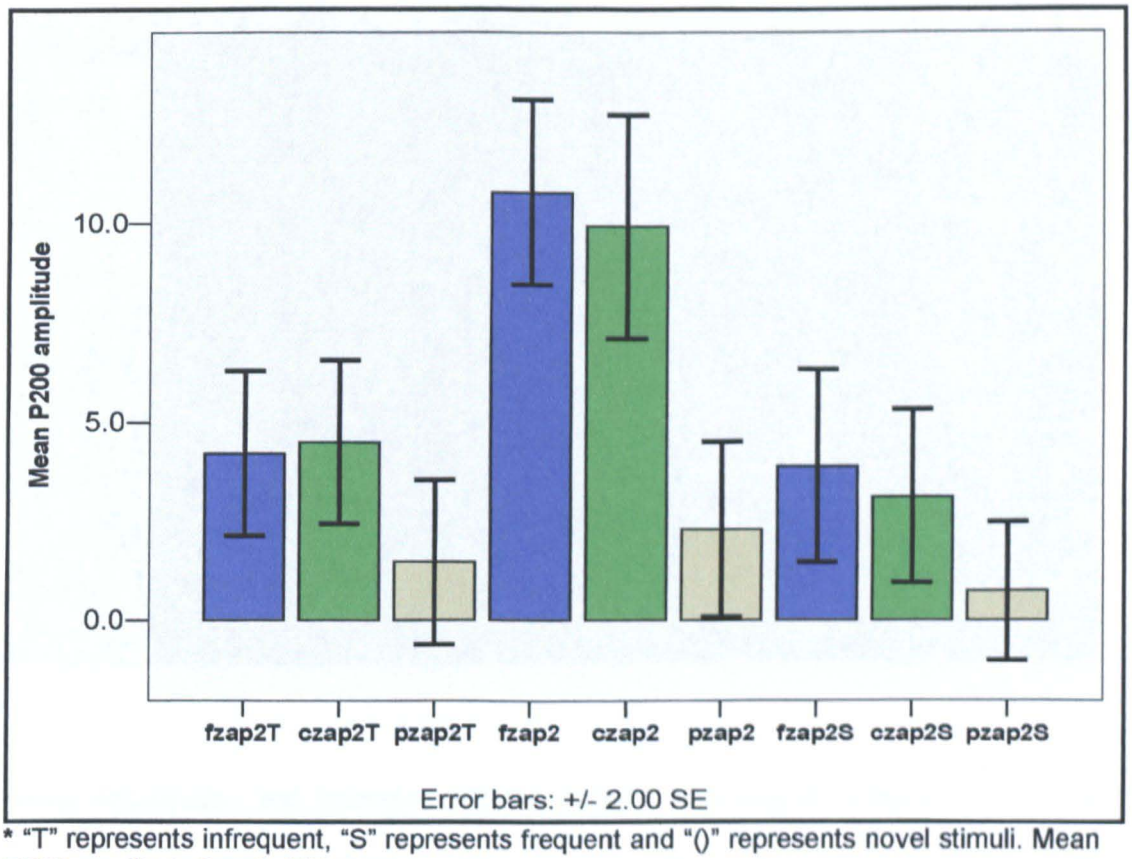
Figure 8.3: P200 latency: stimulus by electrode



* "T" represents infrequent, "S" represents frequent and "()" represents novel stimuli. Mean P200 latencies to auditory stimulus at Cz.

Amplitude: The P200 amplitude to the novel stimulus was significantly greater than that of the frequent and infrequent stimulus (main effect of Stimulus [F (2, 142) = 10.30, p< 0.001]). The P200 peak was greatest in the frontal brain sites and decrease posteriorly (main effect of Electrode [F (2, 142) = 29.99, p< 0.001]). There was a significant interaction of Electrode by Stimulus [F (4, 284) = 4.583, p= 0.003]. This appeared to be due to significantly larger P200 amplitude to the novel stimulus at the fronto-central electrodes compared to Pz (Figure 8.4). There was a significant interaction of Stimulus by Diagnosis [F (2, 142) = 3.373, p= 0.039] due to larger amplitude to the novel than the infrequent stimuli in HIV infected children.

Figure 8.4: Effect of stimulus by electrode interaction on P200 amplitude*

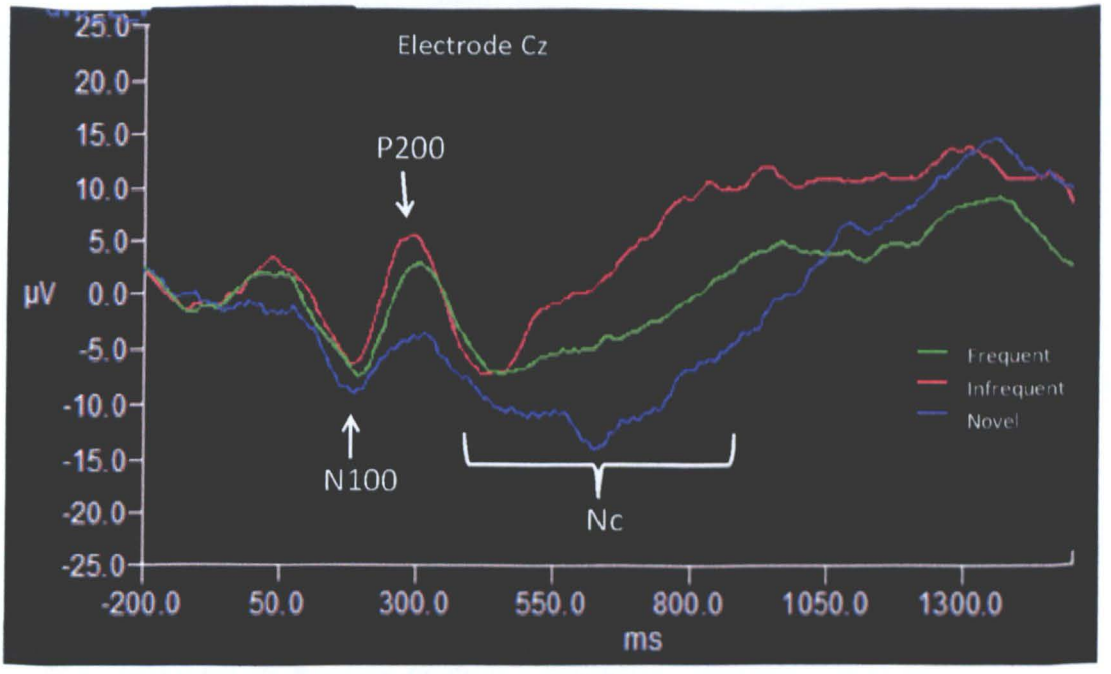


* "T" represents infrequent, "S" represents frequent and "()" represents novel stimuli. Mean P200 amplitude for all children.

8.3.2. Visual ERP results

The visual N100 was defined as the most negative peak between 100-250 msec, while P200 (P250) was defined as the most positive peak between 250-350 msec. The negative component, Nc, was defined as the average amplitude between 400 and 500 msec (Figure 8.5).

Figure 8.5: Grand averages for visual waveforms for controls at Cz



Mean amplitudes and latencies for the defined components were analysed and results have been summarised in table 8.4 below. The amplitude of the visual N100 and Nc components for 18-24 months old children exposed to HIV were significantly smaller than those of controls.

Table 8.4: Mean visual components by age and diagnosis in the HIV study

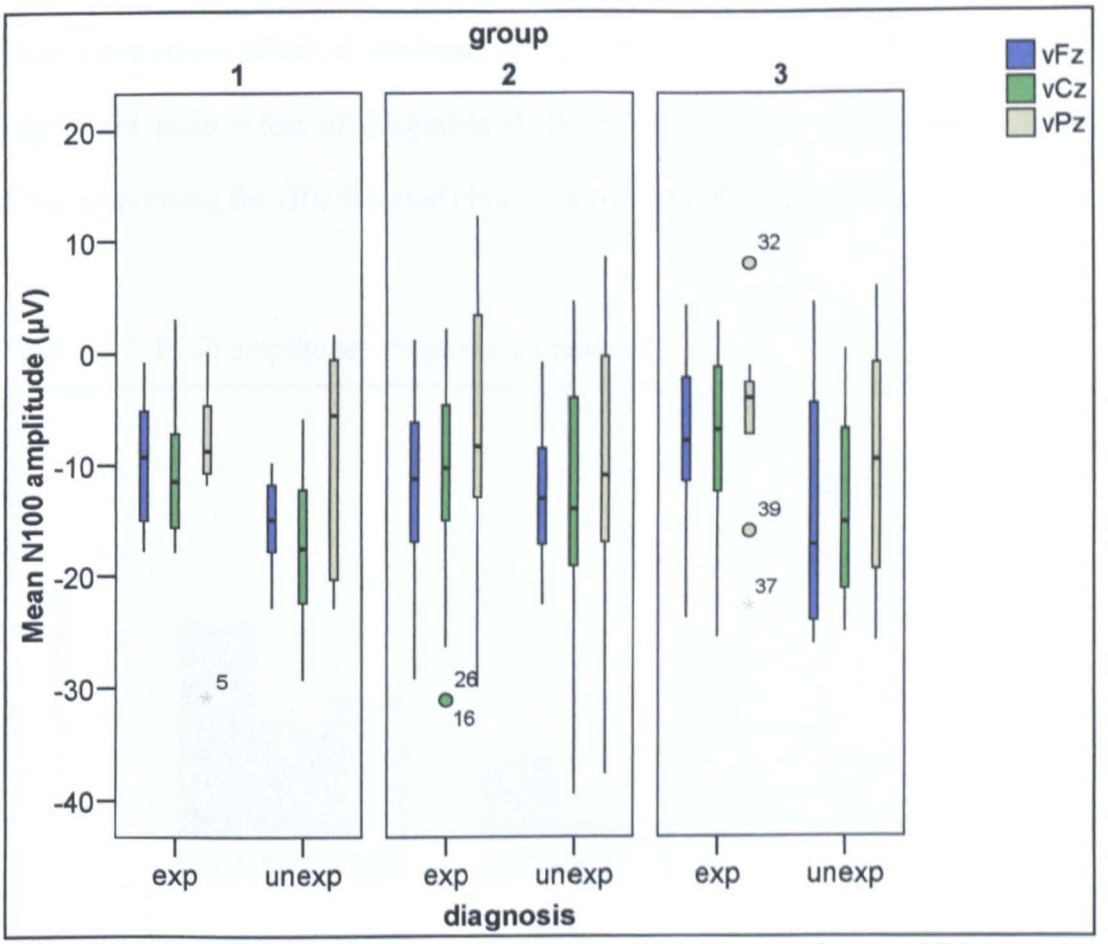
Latency (msec)		18 - 24 months				25 - 29 months				30 - 40 months			
		HIV infected		Controls		HIV infected		Controls		HIV infected		Controls	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
N100	vFz	170.3	39.4	201.0	31.3	182.8	49.0	180.2	33.0	188.9	36.2	174.2	40.0
	vCz	191.1	34.5	182.8	28.3	172.5	43.4	170.7	38.6	205.1	37.2	202.2	35.6
	vPz	167.7	45.2	215.8	50.2	194.1	52.8	182.4	58.9	184.7	66.7	217.2	35.4
P200	vFz	281.7	31.4	291.5	34.4	296.3	40.9	307.2	31.5	307.3	44.1	288.4	29.7
	vCz	282.0	35.1	271.0	23.0	291.2	47.0	297.9	35.0	297.6	40.8	275.4	23.3
	vPz	292.9	45.8	289.0	37.0	295.5	36.8	310.9	38.1	322.4	35.0	299.6	32.8
Amplitude (μV)													
N100	vFz	**-9.7***	6.4	-15.2	4.3	-11.3	7.6	-12.1	6.0	-9.0	8.0	-14.0	10.8
	vCz	**-10.3***	7.2	-17.5	7.5	-11.5	10.0	-12.7	10.4	-9.0	8.4	-14.3	8.6
	vPz	-10.0	10.0	-9.3	10.5	-7.3	12.0	-8.7	11.7	-7.4	7.2	-9.6	11.1
P200	vFz	13.0	18.2	-5.8	7.9	-1.8	8.8	-0.1	10.2	1.5	4.6	-0.5	11.5
	vCz	5.0	10.7	-8.1	5.9	-2.6	11.9	-0.9	11.5	-3.0	8.4	-5.0	12.2
	vPz	0.7	11.5	-6.5	10.2	2.6	12.1	0.0	11.6	-1.7	7.2	-3.2	12.2
Nc	vFz	**-7.1***	14.0	-11.3	12.6	1.2	15.1	-0.4	14.1	**-3.9***	13.8	-1.6	16.5
	vCz	**-3.7***	11.2	-20.1	12.4	-12.7	18.3	-10.0	7.2	-3.7	8.7	-15.5	12.0
	vPz	-10.1	3.1	-21.9	11.6	-12.2	15.2	-5.7	10.4	-7.3	12.4	-15.0	11.3

8.3.2.1 *VISUAL N100*

Latency: The analysis of the N100 latency using repeated-measures ANOVA revealed a significant main effect of Electrode [F (2, 138) = 5.438, p= 0.005] due to a longer latency at Pz compared to fronto-central electrodes. There was an interaction effect of Electrode by Diagnosis by Age [F (4, 138) = 2.936, p= 0.030]. *Post hoc* analyses did not reveal any significant differences of the visual N100 latency due to both age and diagnosis.

Amplitude: The visual N100 amplitude to the novel stimulus was significantly larger than that of the frequent stimuli (main effect of Stimuli [F (2, 138) = 3.722, p= 0.029]. The N100 amplitude was also larger in the fronto-central electrodes than in the posterior (main effect of Electrode [F (2, 138) = 11.516, p< 0.001]. There was also a main effect Diagnosis [F (1, 69) = 5.297, p= 0.024] due to smaller N100 amplitudes for HIV-infected children compared to community controls in the fronto-central electrodes (Figure 8.6).

Figure 8.6: Boxplot showing effect of diagnosis on N100 amplitude*



*Mean N100 amplitudes to visual stimuli at midline electrodes. "exp" means HIV positive and "unexp" are controls. Mean N100 amplitudes averaged for all stimuli. Group 1 is age 18-24 months, group 2 is 25-29 months and group 3 is 30-40 months. "vFz" represents visual N100 at Fz.

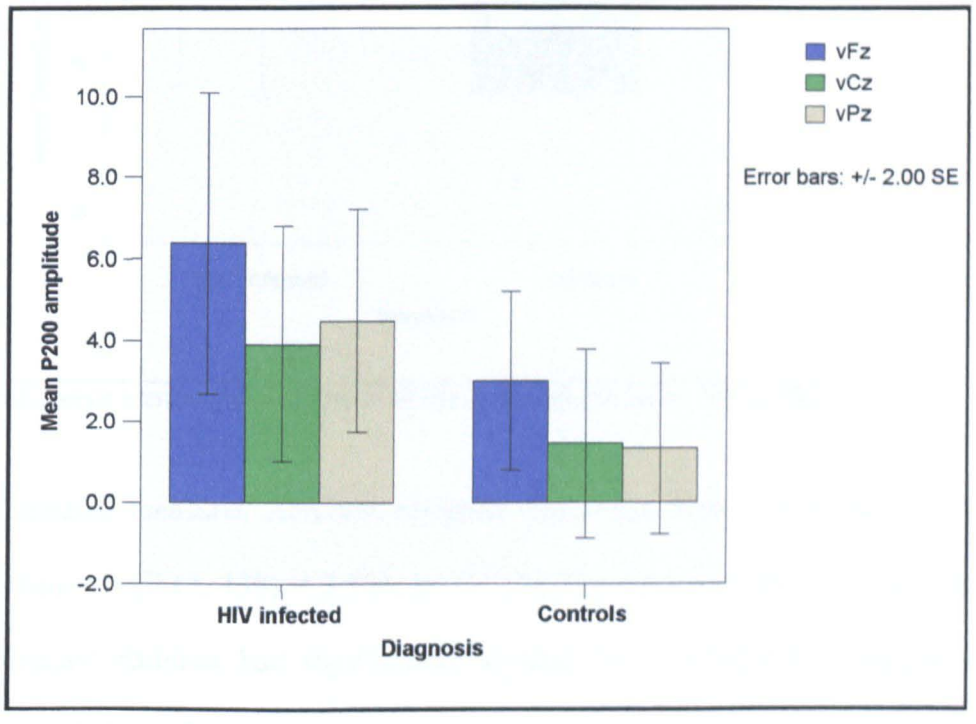
8.3.2.2 *VISUAL P200*

Latency: A repeated-measures ANOVA of the latencies of P200 reveals a significant main effect of Stimulus [$F(2, 138) = 11.516$, $p < 0.001$] due to a significantly shorter latency of the novel compared to the frequent stimuli. There was a significant interaction of Stimulus by Age [$F(4, 138) = 2.957$, $p = 0.031$] but *post hoc* analysis did not reveal any significant differences dependent on age.

Amplitude: The P200 amplitude to the novel stimulus was significantly smaller than that of the frequent or infrequent (main effect of Stimulus [$F(2, 136) = 7.014$, $p <$

0.003]). The amplitude was largest in the frontal electrode and decreased posteriorly (main effect of Electrode [F (2, 136) = 4.534, p= 0.018]). There was a significant main effect of Diagnosis [F (1, 68) = 5.481, p= 0.022] due to larger P200 amplitudes for HIV infected children than controls (Figure 8.7).

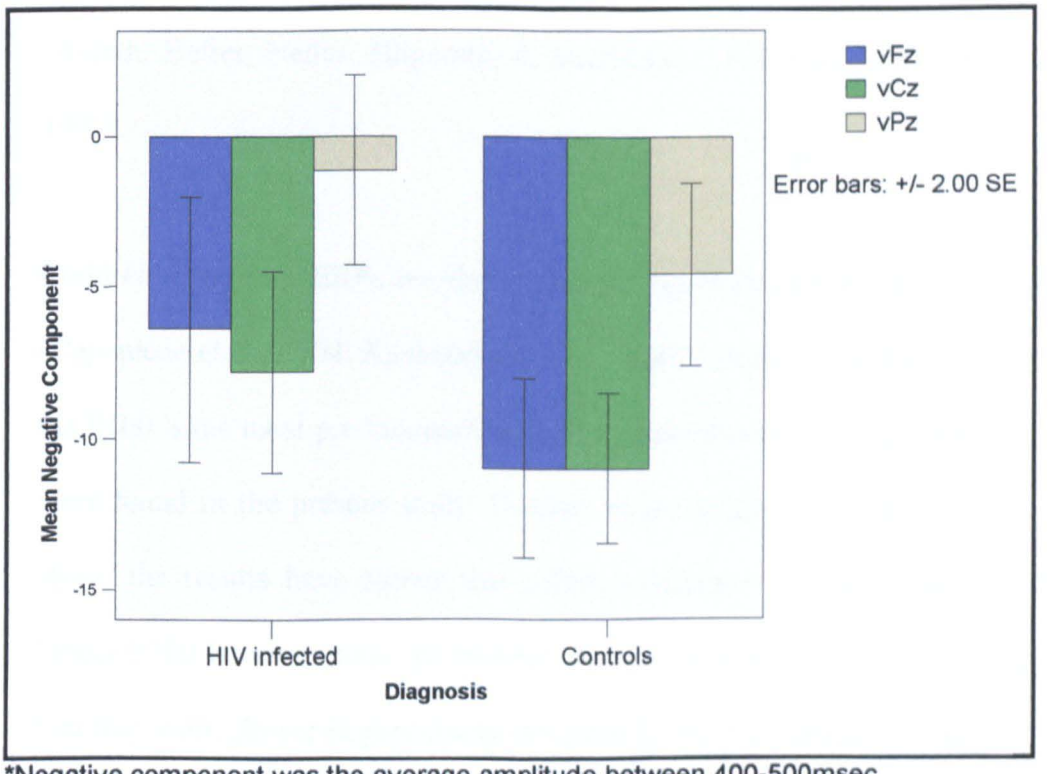
Figure 8.7: P200 amplitude: diagnosis by electrode



8.3.2.3 *NEGATIVE COMPONENT, Nc*

The amplitude of the negative component, Nc was largest in the fronto-central electrodes (main effect of Electrode [F (2, 138) = 18.268, p< 0.001]) than at the posterior site. There was also a significant main effect of Diagnosis [F (1, 69) = 6.528, p= 0.013] that occurred due to smaller mean Nc amplitudes for HIV infected children compared to study controls (Figure 8.8).

Figure 8.8: Mean negative component*: diagnosis by electrode



*Negative component was the average amplitude between 400-500msec

Repeated measures ANOVA revealed interaction effects of electrode by Sex by Diagnosis [F (4, 138) = 3.405, p= 0.013]. *Post hoc* analyses showed that the HIV infected children had significantly smaller Nc compared to controls at fronto-central electrodes.

8.4. Discussion

The aims of the study were to examine the extent to which the neural responses to novelty in the visual and auditory paradigms differed in patients infected with HIV compared to controls. The use of event related potentials may provide supporting evidence of the differences in cognitive outcomes of children infected with HIV compared to community controls. While results from adult studies using ERPs have shown differing results (Baldeweg et al., 1993; Connolly et al., 1994; Goodin, Aminoff, Chernoff, & Hollander, 1990; McAllister et al., 1992), those with HIV

have ERPs that have prolonged latencies (Goodin et al., 1990), reduced amplitudes (Arendt, Hefter, Nelles, Hilperath, & Strohmeyer, 1993) or both (Baldeweg et al., 1993).

Children's auditory ERPs are dominated by the P100 and N200 (or N250) peaks (Ceponiene et al., 2004; Kushnerenko et al., 2002). In early childhood (1 – 4 years), the P100 is the most predominant peak (Kushnerenko et al., 2002). Similar findings were found in the present study. Further, in the auditory paradigm of the present study, the results have shown that children infected with HIV had significantly longer P100 latencies after 30 months of age than community controls suggesting that they were slower in processing the sounds than the controls. A number of ERP studies investigating the development of auditory sensory systems have shown that peak latencies of P100 and N200 decrease with increasing age (Goodin, Squires, & Starr, 1978; Paetau, Ahonen, Salonen, & Sams, 1995; Ponton et al., 2000) due to maturational processes. This suggests that children with prolonged P100 and N200 latencies are cognitively impaired compared to those with shorter latencies. In the present study, the N200 component was not studied since it was not present in many children.

In the visual paradigm, all the components studied (i.e. N100, P200 and Nc) differentiated between the HIV infected children and community controls. The N100 amplitudes of HIV infected children were significantly smaller than those of community controls. The increased amplitude is attributed to enhanced processing (Key et al., 2005) and probably controls had better processing that infected children as they had larger amplitudes. The amplitude of the visual P200 component

increases with complexity of the stimuli (Pernet et al., 2003). In the present study, children infected with HIV had significantly larger P200 amplitudes than controls. Since the P200 amplitude increases with complexity, we propose that perhaps the HIV infected children found the abstract art used as novel stimulus more complex than the controls did. The Nc is associated with general attentive and alerting response to stimuli (Richards, 2003). While the Nc was present in both groups, it was significantly larger in the control group further supporting the hypothesis suggesting that the HIV infected children may have cognitive impairment. The Nc has been observed in response to visual novel stimuli with a frontal topography (Courchesne, 1990). The amplitudes of this frontally maximal Nc components have been found in other studies to be largest in young children and to decrease with age (Courchesne, 1978; Gumenyuk, 2005). The effect of age was not found in the present study possibly due to the small age range in the study sample.

The patterns of abnormalities show that children with HIV infection are likely to have delayed maturation, slower processing and less attentive compared to their matched controls. This may impair normal psychological development and academic outcomes. Neuropsychological studies have shown that HIV positive children are impaired cognitively, developmentally, emotionally, psychologically, behaviourally and educationally (Bisiacchi et al., 2000; Coplan et al., 1998; Nozyce et al., 2006; Wolters et al., 1997). The results of the present study showed that children with HIV infection and community controls had different auditory and visual ERPs suggesting the usefulness of ERPs in the study of pre-school children in a rural environment with exposure to HIV.

CHAPTER 9

General Discussion

9.1 Introduction

Malaria, pneumococcal meningitis and HIV are caused by different organisms: a parasite, bacteria and virus respectively. These diseases have a propensity of attacking the central nervous system causing neurological deficits and cognitive impairment. Children under 5-years old bear the brunt of these infections and face death or life-long consequences as a result. In SSA, the morbid consequences of infection are further complicated by other social and environmental factors including malnutrition, poverty, lack of schooling opportunities, conflicts/wars, orphanhood in the case of HIV, and a general lack of adequate health facilities (Grantham-McGregor et al., 2007). Those who survive may have impaired development and struggle at school and this may result in poor national growth and economic burden.

The pathogeneses of these infections are different. In falciparum malaria, the parasite is confined to the red blood cells mostly within blood vessels. The infected red blood cells, in the later stages, become sequestered within the vasculature of the brain, but do not invade the parenchyma of the brain. The sequestration occurs in both the gray and white matter throughout the brain, giving rise to a diffuse encephalopathy. In acute bacterial meningitis (ABM), the pathology involves the covering of the brain (meninges), with vasculitis in the vessels as they cross the meninges. The blood brain barrier is impaired, giving rise to cerebral oedema and

raised intracranial pressure. Brain damage is often focal, around the vessels affected by the vaculitis, particularly affecting the grey matter. Human immunodeficiency virus infects lymphocytes, gains access to the brain parenchyma, with infection of the microglia and to a lesser extent astroglia. The white matter appears to be particularly affected. Thus these three common conditions may provide insights into different mechanisms of brain damage.

Cognitive abnormalities as defined by neuropsychological batteries occur in children exposed to HIV (Chase et al., 2000; R. Smith et al., 2000; Wolters et al., 1997), severe falciparum malaria (Boivin, 2002; Carter, Mung'ala-Odera et al., 2005; Carter, Neville et al., 2003; Carter, Ross et al., 2005; Holding et al., 1999; Holding et al., 2004; Kihara et al., 2006) or ABM (Grimwood et al., 2000; Schmidt et al., 2006; H. G. Taylor et al., 1998). The use of adapted Western neuropsychological tests in SSA continues as psychologists strive to develop more appropriate tests to detect subclinical changes and thus assess the impact of these conditions on children in the region.

There exists a burden of disease in resource poor settings but lack of standardized testing tools has limited the efforts to determine its extent. This study investigated the usefulness of ERPs as electrophysiological marker of cognitive performance in children in a rural setting. While a similar study has not been reported in the region, the results of the present study suggest possible usefulness of ERPs as surrogate markers of cognition. The results are however interpreted with caution due to lack of a gold standard with which to compare the ERP results with. Many authors have suggested that ERPs may provide a more sensitive indicator of early cognitive

impairments (Courchesne, 1978; Ollo, Johnson, & Grafman, 1991). The main advantage of ERPs is that they provide non-invasive information about brain activity with a high time resolution (Gumenyuk et al., 2004) and do not necessarily require the participants to provide motor or verbal response (Key et al., 2005).

9.2 Summary of event related potential and brain insults

The aims of this thesis as stated in chapter 4 were to demonstrate that ERPs are associated with neurocognitive impairment following the most common CNS infections affecting children in sub-Saharan Africa, namely falciparum malaria, ABM and HIV .

9.2.1 ERPs and falciparum malaria

In the examination of 50 children aged 6-7 years old with a history of severe falciparum malaria and age-matched controls, the results showed that children exposed to CM, M/S and PM had significantly longer N200 latencies than the controls. Further, the children exposed to PM and M/S had smaller N200 amplitudes than controls. The P3a latency of children with a history of CM and M/S were significantly longer, while the P3a amplitude for children with a history of M/S was shorter than that of the community controls. In the visual paradigm, children exposed to M/S had significantly longer P200 latencies and shorter P200 amplitudes than community controls.

These findings suggest that children with a history of severe falciparum malaria had an abnormal response to novel stimuli ERPs compared to controls. The components affected most were the auditory N200, P3a and visual P200. The auditory N200

component is detected when there is a deviation from the expected stimuli (Naatanen & Picton, 1986), and is thought to be generated by the frontal and parietal cortical areas (Gomot et al., 2000) and the Heschl's gyrus (Takeshita et al., 2002). These areas of the brain are responsible for language and higher order processing activities such as attention and decision making. The P3a from an unattended paradigm is thought to reflect involuntary attention as well as inhibition and its sources are in the prefrontal cortex (R. T. Knight, 1991).

The review of literature on the effects of falciparum malaria on cognition showed that its effects on brain are not focal (Kihara et al., 2006). Previous research has shown that severe falciparum malaria affects speech and language (Carter, Mung'ala-Odera et al., 2005; Carter, Murira et al., 2003), attention (Boivin, 2002; Holding et al., 2004) and planning/executive functions (Carter, Ross et al., 2005; Holding et al., 2004), a result that is suggested by ERP results of the present study.

9.2.2 ERPs and pneumococcal meningitis

The study of sixty-five children aged between 4-15 years old exposed to pneumococcal meningitis and age-matched controls showed that children with a history of pneumococcal meningitis had significantly longer P100 latencies and smaller N200 amplitudes than age-matched peers in the auditory paradigm. The P3a amplitude of children with a history of pneumococcal meningitis remained constant throughout the 4-15 years but that of unexposed children increased with increasing age. In the visual paradigm, children exposed to pneumococcal meningitis had longer N100 and P200 latencies at the frontal and central electrodes than controls.

The P100 component is usually interpreted as an indicator of preferential attention to sensory inputs and is thought to reflect one's level of arousal (Key et al., 2005). The results suggest that children with a history of pneumococcal meningitis may have had lower arousal levels and perhaps were less attentive to the auditory task. Poorer attention among children with a history of pneumococcal meningitis may have resulted in smaller auditory N200 amplitudes. The shorter visual N100 latencies could be related to subtle impairments in visual processing while shorter visual P200 latencies could be attributed to impairment to higher level processing.

Previous research has reported cognitive deficiencies after bacterial meningitis in children including difficulties in learning, deficits in short-term memory, behavioural problems and poorer academic performance (Grimwood et al., 2000) up to twelve years post infection.

9.2.3 ERPs and human immune-deficiency virus

In the study of forty children HIV+ aged between 18-40 months and age-matched controls, the results showed HIV infected children had longer P100 latencies, larger P200 amplitudes and smaller mean Nc amplitudes (in those between 30-40 months).

The majority of children with HIV infection have central nervous system abnormalities but the exact relationship between exposure and outcome appears to depend upon age, biological and environmental risk factors. Not much is known of the nature of these relationships outside of Resource Rich Countries, and of the longer-term consequences. The results of the present study suggest that the HIV-

infected children had more attention problems than their peers, which is supported by neuropsychological studies that have found attention deficit hyperactive disorder in children infected with HIV (Watkins et al., 2000).

9.3 Implications of results of the study

The present study evaluated groups of children who had been exposed to severe falciparum malaria (i.e. cerebral malaria (CM), malaria plus seizures (M/S) or prostrate malaria (PM)), pneumococcal meningitis or were infected with the human immune-deficiency virus (HIV). Auditory and visual ERPs paradigms distinguished the groups exposed to these infections from the controls (Table 9.1 and 9.2). However, in the absence of a gold-standard measure, it is impossible to attribute the differences to impaired cognition. In sub-Saharan Africa, there lacks standardized neuropsychological tests and future research should seek to fill this gap. The pattern of the ERP abnormalities differed depending on stimuli presented and disease group.

In the auditory paradigm, the early P100 component distinguished between children exposed to PM and HIV and community controls but not those exposed to severe falciparum malaria. The N200 component distinguished all exposed children from community controls. It was however not studied in HIV+ children since they were young. The P3a (and P200 for HIV) component distinguished between cases and controls (Table 9.1).

Table 9.1: Summary auditory ERP components of cases and controls

Auditory Stimulus		Falciparum Malaria M/S	Falciparum Malaria PM	Falciparum Malaria CM	Pneumococcal Meningitis	Human Immuno-deficiency Virus
P100	latency	X	X	X	↑	↑
	amplitude	X	X	X	X	X
N200	latency	↑	↑	↑	↑	NE
	amplitude	↓	↓	X	X	NE
P3a	latency	X	X	↑	X	↑
	amplitude	↓	X	↓	X	↓

↑ = The component differentiated between exposed children and controls and latency was significantly longer; ↓ = Component differentiated between cases and controls and amplitudes were shorter; **X** = the component did not differentiate; **NE** = component not examined

The significant differences in the auditory P100 components in children exposed to pneumococcal meningitis and HIV infections may suggest a reflection of an impairment of early "exogenous" ERP component. Exogenous components are elicited by sensory stimuli and represent activity at the periphery sensory system (Key et al., 2005). Previous research has shown that ABM causes hearing impairment through damage to the cochlear (Bedford et al., 2001; Pikis et al., 1996; Winter et al., 1997). HIV-positive patients have an increased incidence of both conductive and sensorineural hearing loss (Madriz & Herrera, 1995). The longer auditory P100 latencies in children in the present study could have resulted from subtle hearing loss resulting from pneumococcal meningitis or HIV. Children with a history of pneumococcal meningitis had smaller visual P100 amplitudes than their unexposed peers. The P100 component increases with age and has been suggested to be an indicator of maturation (Ponton et al., 2000). The result suggests that

children exposed to Pneumococcal meningitis may have delayed development compared to controls. Children with a history of severe falciparum malaria had normal auditory P100 components but their later "endogenous" ERP components were significantly longer than those of their unexposed peers. Endogenous components represent cortical activity and depend on psychological variables such as attention or task relevance. These components have been reported to represent cognitive process.

In the visual paradigms, some significant differences were found between P200 and Nc components of children exposed to falciparum malaria, pneumococcal meningitis, HIV compared to age-matched controls (Table 9.2) but the abnormalities of latencies and amplitudes differed between the conditions. No differences were detected in N100 in any of these groups.

Table 9.2: ERP components distinguishing cases and controls in the visual paradigm

Visual Stimulus		Falciparum Malaria M/S	Falciparum Malaria PM	Falciparum Malaria CM	Pneumococcal Meningitis	Human Immuno-deficiency Virus
N100	latency	X	X	X	X	X
	amplitude	X	X	X	X	X
P200	latency	X	X	X	↑	↑
	amplitude	↓	↓	X	X	X
Nc	Mean amplitude	X	↓	X	X	↓

↑ = The component differentiated between exposed children and controls and latency was significantly longer; ↓ = Component differentiated between cases and controls and amplitudes were shorter; X = the component did not differentiate

The P200 latency was significantly longer in children exposed to pneumococcal meningitis and HIV infection compared to controls (Table 9.2), whilst the P200 amplitude was smaller in children admitted with malaria plus seizures or prostate malaria, but not cerebral malaria. The amplitude of the visual P200 component is an index of ability to comprehend complex stimuli while the latency is thought to index mechanisms of selective attention and feature detection processes (Hackley et al., 1990; Luck & Hillyard, 1994).

Children exposed to HIV and prostate malaria had smaller visual Nc amplitudes than their age-matched peers. The Nc represents the child's allocation of attention to novelty. The finding in prostate malaria may be a chance finding, since no significant abnormalities were found in the more severe form of malaria i.e. cerebral malaria.

9.4 Limitations of the study

These study results need to be interpreted taking into consideration the following limitations:

1. The lack of standardization of ERP paradigms around the world reduces useful comparison. In particular, the interstimulus interval, intensity of sound, types of stimuli, types of electrodes, reference electrodes used, data capture and amplification differ from laboratory to laboratory. The comparison of results of studies with fundamental differences is problematic.
2. It remains difficult to attribute effects of *P. falciparum*, particularly in malaria endemic areas, to the exclusion of other causes. A study in Malawi found that other causes resulted in 24% of those that died with a clinical definition of CM at post-mortem (T. E. Taylor et al., 2004). Similarly, children with HIV infection are susceptible to other infections and thus it is difficult to attribute their poor performance to HIV alone. The classification of children in the malaria and meningitis studies was obtained retrospectively from the hospital database, and other co-morbidities could not be excluded. It remains a challenge in SSA to attribute a child's developmental impairment to a single condition in the presence of the range of insults to which these children are exposed. Effort was made to exclude children with other causes of neurological impairment other than the ones under investigation but this did not exclude those with other possible causes of cognitive impairment such as poor nutrition, children born with low-birth weight and those living in un-enriched environments.

3. There are challenges of adopting Western neuropsychological tests in non-Western contexts due to lack of familiarity with test material and test demands. There is a possibility ERPs in the rural children caused anxiety and the results obtained may be more a reflection of other psychological factors other than cognitive performance. While the refusal rates were very low, almost non-existent, that none of the groups, whether those under investigation or the controls, had any prior contact with the equipment and hence no group had undue advantage.
4. A proportion of children exposed to pneumococcal meningitis suffer from hearing loss. Efforts were made to test hearing before the ERP experiments but there is a possibility that children exposed to pneumococcal meningitis performed differently in the auditory paradigm due to subtle memory loss other than CNS deficits.
5. In the present studies, the duration between the time of insult and assessment was not analyzed. Previous studies have suggested progressive deterioration of cognitive performance over time (Bedford et al., 2001; Grimwood et al., 2000) and possibly this would have been examined if we included this variable in the model.

9.5 Suggestions for future research

I have established that ERPs can be performed in rural Africa and can be used to examine children in without access to computers or schooling. Their application on infant populations will encourage more research on this group which is often excluded due to lack of appropriate tools. Future research should focus on infants

and children in SSA facing brain insults as a result of conditions such as neonatal jaundice, premature births, sickle-cell disease, epilepsy etc.

There is need for longitudinal studies to determine whether exposure to brain insults results in progressive decline of cognitive abilities. The duration since the time of insult can be used to determine the effects of time on cognitive performance.

The trends in the comparative analysis of the ERPs components of children with different brain insults showed different outcomes (section 9.3). Since these insults had different pathophysiologies, future studies should focus on the relationship of the neural origins of the various ERP components and neuropsychological outcomes.

The passive ERP paradigms were found to be useful in our setting. I hope to use this paradigm in a group of children with epilepsy to determine the effects of disease and drug usage on the ERP components and compare with community controls. The passive paradigm may prove useful among this group since there are many uncooperative or sedated children whom we can test.

9.6 Concluding remarks

The ERP technique of measuring and detecting cognitive deficits in children of different ages is useful in SSA. The passive (non-attending) paradigm can be applied to children regardless of age, providing the maturational changes to ERPs by having appropriate controls. The ability to use ERPs on infants and pre-verbal

children will enhance early determination of the effects of insults during infancy on cognition and hopefully an intervention can be instigated earlier.

The results suggest that the CNS infections may result in neuro-developmental delays in childhood, which may manifest as executive dysfunction (poor working memory) later in life. Further, CNS infections may interfere with normal education outcomes by precipitating attention deficit amongst children post infection. The results suggest that these serious infections, falciparum malaria, pneumococcal meningitis and HIV present a major public health burden.

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Appendix 1

The Ten Questions Questionnaire (TQQ)

Child's Personal Details

Today's Date: ..... [__][__]/[__][__]/[__][__][__][__]

TQ Number: ..... [__][__][__][__][__]

Child's Name: _____

Mother's Name: _____

EZHHID: ..... [__][__][__][__][__][__][__][__]

RESID: ..... [__][__][__][__][__]

Child's DOB: ..... [__][__]/[__][__]/[__][__][__][__]

Child's Age: ..... [__][__]

Child's Sex: ..... [__]

Child's place of Birth: ..... [__]
Does the child attend school regularly? (Y/N)..... [__]

Who will answer questions about the child? ..... [__]

1. The child's mother.
2. The child's father.
3. The child's grandmother.
4. The child's sibling.
5. Another relative.
6. Other.

Is the informant one who mainly takes care of the child?(Y/N) .....[__]

Fieldworker Code.....[__]

Child's EZHHID: [__][__][__][__][__][__][__]

Ten Questions

Question 1. **Compared with other children, did the child have any serious delay in sitting, standing or walking?** YES [__]* NO[__]

Kuhalanisha na ahoho angine, yuno mwanao <u>Watoa sana</u> kukeresi, kuima koko hedu kunenda?

Question 2. **Compared with other children doe the child have difficulty seeing, either in the daytime or at night?** YES [__]* NO [__]

Kuhalanisha na ahoho angine, yuno muhoho ana thabu ya kuona;
a] mtsana

b] hedu usiku?(kala kaona, mpaka agwirwe mkono?)

Question 3. **Does the child appear to have difficulty hearing?** YES [__]* NO [__]

Yuno mwanao anathabu ya kusikira (hedu ana masikiro maziho)

Question 4. **When you tell the child to do something, does he/she seem to understand what you are saying?** YES [__] NO [__]*

Ukimwambira utu, yuno mwanao nikuelewa zho udzizho nena?

Question 5. **Does the child have difficulty in walking or moving his/her arms or does he/she have weakness and/ or stiffness in the arms or legs?** YES [__]* NO [__]

Child's EZHHID: [__][__][__][__][__][__][__][__]

Yuno muhoho anathabu ya kunenda hedu kuusa
mikono hedu kukosa nguvu na/ hedu kumalala
mikono hedu magulu?

Question 6. **Does the child sometimes have fits, become rigid,**
Or lose consciousness? YES [__]* NO [__]

Yuno mwanao nikufitika wakathi mungine akamalala
hedu kungamiza fahamu?

Question 7. **Does the child learn to do things**
like other children his/her age? YES [__] NO [__]*

Yuno mwanao nikudzifundisha kuhenda
mautu here ahoho a marikage (here viryahu, kuheka madzi, kurisa hedu kushera)?

Question 8. **Does the child speak at all (can he/she make himself/**
herself understood in words: can he/she say any
recognizable words)? YES [__] NO [__]*

Yuno mwanao anadima kunena (anadima kunena
akaeleweka: anadima kunena maneno ga kumanyikana)?

Child's EZHHID: [__][__][__][__][__][__][__]

Question 9. **Is the child's speech in any way different from normal (not clear enough to be understood by people they don't talk to often)?**

YES [__]* NO [__]

Kuno kunenakwe yuno mwanao kunatofauti yoyosi (nikukala kaeleweka kwa atu ambao kamanena mara kwa mara) hedu ana kitsembe ama shida nyingine yoyosi ya kunena?

Question 10. **Compared with other children of his/her age does the child appear in any way mentally backward, dull or slow?** YES [__]* NO [__]

Kuhalanisha na ahoho angine a rikare yuno mwanao anathabu ya akili punguani, uzuzu hedu a goigoi?

Does the child have any serious health problem not yet mentioned?

YES [__] NO [__]

Yuno muhoho anathabu yoyosi ya kiafya iriyo kafwihadzire?

If yes, write down what kind of problem it is:

Interviewer: Answer the question below by circling of the three points. The questionnaire result positive if the response to any one or more of the Ten Questions has an asterisk (*) next to it. If no response has * next to it, then the result is negative.

Should this child be referred for professional evaluation?

YES [__] NO [__]

1. No, because the questionnaire result is negative
   And there is no **X** in the box below.

2. Yes, because, although the questionnaire result is negative,
   There is an **X** in the box below.

3. Yes, because the questionnaire result is positive.

For data entry only:

Does the box below contain an **X**? YES [__] NO [__]

Information sheet

(Explanation to be given verbally and in writing in an appropriate language)

Study Title: The use of Event Related Potentials to measure visual and auditory processing mechanisms in cognitively impaired children

Lead Investigator: Michael Kihara, Research Fellow, KEMRI/Wellcome Trust

We are from KEMRI!

WHAT DOES KEMRI DO?

KEMRI is a research institution, which aims to learn more about health issues in Kenya in order to improve prevention and treatment for everybody in the future. One of KEMRI's current interests is to learn more about the effects of infections that can affect the brain and leave a child with learning problems.

WHAT IS THE PURPOSE OF THIS STUDY?

Research studies in Kilifi show that approximately one out of four children who have suffered from a brain infection is left with learning problems. We are trying to find a way of identifying these children early. If we can identify them early, we can begin to plan to give them appropriate help.

WHAT ARE WE REQUESTING FROM YOU?

If you have a son/daughter who is less than 9 years old and has, in the past or at present, been admitted to a hospital following severe malaria or meningitis, we would like to include him/her in our study. Bring your child to an appointment at the Assessment Centre in the Kilifi District Hospital grounds. The appointment will last approximately two and a half hours.

If you agree that he/she participate in the study, your child will perform a test called electroencephalography (EEG) (Show picture). She/he will wear a hat made of leads which can read/measure how she/he processes pictures and sounds. This procedure will take a maximum of 90 minutes per child. We would like to repeat the same procedure after 24 months to monitor your child's progress.

For children above 6 years, we will ask them to undergo some activities measuring their memory, attention and planning.

After a child reaches her/his sixth birthday, we would like to see you again. Your child will then be asked to undergo the some activities to look at their learning development.

WHAT ARE THE RISKS OF TAKING PART?
Your child will NOT be asked to take any medicine or injections. Our tests can be enjoyable and are free of any known risk.

WHAT ARE THE BENEFITS OF TAKING PART?
If we are concerned about your child's test results, we will discuss with you and advise you on available services. The study could ally fears that the childhood infection left traces of learning impairment.

WHAT IF YOU DECIDE NOT TO TAKE PART OR CHANGE YOUR MIND?
You are free to decline consent for your child to participate in the study. If you decide to consent now and later change your mind, you are free to drop out of the study at any point.

If you require further information please contact Mr. Kihara at the KEMRI centre, Kilifi. (Office in Admin section 041 525453, mobile 0722805290)

WHAT WILL HAPPEN ONCE THE STUDY'S OVER?
The parents will be given information about their children's general learning abilities, and where there seems to be impairment, they will be advised on the community resources that are available.

Consent form

(Explanation to be given verbally and form used in an appropriate language)

Study Title: **The use of Event Related Potentials to measure visual and auditory processing mechanisms in cognitively impaired children**

Lead Investigator: Michael Kihara, Research Fellow KEMRI/Wellcome Trust

When you sign below it shows that you have agreed that your son/daughter and you are part of the study. If you do not understand any part of the information sheet, you are allowed to ask questions. Do not sign until we have answered your questions satisfactorily.

I wish that my son/daughter take part in the study as explained above. I understand that these procedures are safe and will not endanger my son/daughter. I understand that my child is free to withdraw from the study. I understand the results of the study will be confidential, and I may choose to be told or not be told my child's results.

Signature (or thumb print) of volunteer_____

Name in capitals _____

Date _____

I have read the information sheet to _____ (volunteer's name) in a language he understands. I believe that he gives consent to take part in the study.

Signature of translator_____

Name in capitals_____

Date_____

I have witnessed the above being discussed with_____ (volunteer's name) in a language he understands. I believe he gives consent to take part.

Signature of witness_____ (Only required if volunteer unable to read)

Name in capitals_____

Date_____

If you require further information please contact Mr. Kihara at the KEMRI centre, Kilifi. (Office in Admin section
041 525453, mobile 0722805290)

Appendix 3: Information sheet / Consent Form (for controls)

Information sheet

(Explanation to be given verbally and in writing in an appropriate language)

Study Title: The use of Event Related Potentials to measure visual and auditory processing mechanisms in cognitively impaired children

Lead Investigator: Michael Kihara, Research Fellow, KEMRI/Wellcome Trust

We are from KEMRI!

WHAT DOES KEMRI DO?

KEMRI is a research institution, which aims to learn more about health issues in Kenya in order to improve prevention and treatment for everybody in the future. One of KEMRI's current interests is to learn more about the effects of infections that can affect the brain and leave a child with learning problems.

WHAT IS THE PURPOSE OF THIS STUDY?

Research studies in Kilifi show that approximately one out of four children who have suffered from a brain infection is left with learning problems. We are trying to find a way of identifying these children early. If we can identify them early, we can begin to plan to give them appropriate help.

WHAT ARE WE REQUESTING FROM YOU?

If you have a son/daughter who is less than 7 years old, and has **never** been admitted to hospital with severe malaria or meningitis, we would like to include him/her in our study. Bring your child to an appointment at the Assessment Centre in the Kilifi District Hospital grounds. The appointment will last approximately two and a half hours.

If you agree that he/she participate in the study, your child will perform a test called electroencephalography (EEG) (Show picture). She/he will wear a hat made of leads which can read/measure how she/he processes pictures and sounds. This procedure will take a maximum of 90 minutes per child. The procedure is to help us compare your healthy child with those who have suffered from severe malaria and meningitis in the past. We would like to repeat the same procedure after 24 months to monitor progress.

For children above 6 years, we will ask them to undergo some activities measuring their memory, attention and planning.

After a child reaches her/his sixth birthday, we would like to see you again. Your child will then be asked to undergo the some activities to look at their learning development.

WHAT ARE THE RISKS OF TAKING PART?
Your child will NOT be asked to take any medicine or injections. Our tests can be enjoyable and are free of any known risk.

WHAT ARE THE BENEFITS OF TAKING PART?
If we are concerned about your child's test results, we will discuss with you and advise you on available services. The study could ally fears that the childhood infection left traces of learning impairment.

WHAT IF YOU DECIDE NOT TO TAKE PART OR CHANGE YOUR MIND?
You are free to decline consent for your child to participate in the study. If you decide to consent now and later change your mind, you are free to drop out of the study at any point.

If you require further information please contact Mr. Kihara at the KEMRI centre, Kilifi. (Office in Admin section 041 25453, mobile 0722805290)

WHAT WILL HAPPEN ONCE THE STUDY'S OVER?
The parents will be given information about their children's general learning abilities, and where there seems to be impairment, they will be advised on the community resources that are available.

Consent form

(Explanation to be given verbally and form used in an appropriate language)

Study Title: **The use of Event Related Potentials to measure visual and auditory processing mechanisms in cognitively impaired children**

Lead Investigator: Michael Kihara, Research Fellow KEMRI/Wellcome Trust

When you sign below it shows that you have agreed that your son/daughter and you are part of the study. If you do not understand any part of the information sheet, you are allowed to ask questions. Do not sign until we have answered your questions satisfactorily.

I wish that my son/daughter take part in the study as explained above. I understand that these procedures are safe and will not endanger my son/daughter. I understand that my child is free to withdraw from the study. I understand the results of the study will be confidential, and I may choose to be told or not be told my child's results.

Signature (or thumb print) of volunteer_____

Name in capitals _____

Date _____

I have read the information sheet to _____ (volunteer's name) in a language he understands. I believe that he gives consent to take part in the study.

Signature of translator_____

Name in capitals_____

Date_____

I have witnessed the above being discussed with_____ (volunteer's name) in a language he understands. I believe he gives consent to take part.

Signature of witness_____ (Only required if volunteer unable to read)

Name in capitals_____

Date_____

If you require further information please contact Mr. Kihara at the KEMRI centre, Kilifi. (Office in Admin section
041 525453, mobile 0722805290)

Appendix 4: Social Economic Questionnaire

NEURO-COGNITIVE ASSESSMENT STUDY

Socio-Economic Status Questionnaire

Child's Name: _____

Study Number: [**N**][**C**][**A**][ ][ ][ ]

EZHHID: [ ][ ][ ][ ][ ][ ][ ]

Assessor: [ ][ ]

Date of Assessment: [ ][ ]/[ ][ ]/[ ][ ][ ][ ]

Respondent: Mother/ **F**ather/ **O**ther: [ ]

1. Yuno muhoho ana ndugu angahi a ndani mwenga? [ ] (NS)
2. Hano mudzini hana ahoho angahi? [ ] (NC)
3. Yuno muhoho, mameye washoma? (Y/N) [ ] (MS)
   Kala washoma wafikirahi?
   (primary 1-3 = 1; primary 4-8 = 2; secondary = 3; tertiary = 4)[ ] (SR)
4. Yuno mamaye muhoho anamanya kunena kizungu? Y/N [ ] (ME)
5. Yuno babaye ama mutsuma wa muhoho, anahenda kazi yani?
   (subsistence farmer = 1
   large-scale farmer(owns cattle, grows cash crops) = 2
   casual labourer = 3
   small business (no employees) = 4
   large business (has employees)/ profession = 5) [ ] (FO)
6. Munagita mara nyingahi kwa siku? [ ] (MD)
7. Hana siku ambayo kurirye chakurya cha dziloni? [ ] (ND)
   Nirini mwisho urihokosa chakurya cha dziloni? [ ] (LD)
   (within last week = 1
   last month = 2
   more than a month = 3)
8. Here kawaida yenu mupatahi unga?
   (grind at the mill = 1; buy = 2) [ ] (FM)

9. Hano muishiho ni henu mundane? (Y/N) [ ] (OH)
   Muna munda wowosi? (Y/N) [ ] (OL)

10. Nyumba yenu inamadirisha aina yani? [ ] (TW)
    (Glass planes = 1
    wooden = 2
    wire mesh only = 3
    none = 4)
11. Nikukala muna sikiza redio?
    (daily = 1; weekly = 2; irregularly = 3; never = 4) [ ] (LR)

Appendix 5: Hearing Assessment Proforma

NEURO-COGNITIVE ASSESSMENT STUDY

Hearing Assessment

Kamplex Audiometer (PC Werth)

Child's Name: _____

Study Number: [**N**][**C**][**A**][ ][ ][ ]

EZHHID: [ ][ ][ ][ ][ ][ ][ ]

Assessor: [ ][ ]

Date of Assessment: [ ][ ]/[ ][ ]/[ ][ ][ ][ ]

Right Ear: Air Conduction

Frequency:	500HZ	[ ] (RA500)
	1000HZ	[ ] (RA1000)
	2000HZ	[ ] (RA2000)
	4000HZ	[ ] (RA4000)

Left Ear: Air Conduction

Frequency:	500HZ	[ ] (LA500)
	1000HZ	[ ] (LA1000)
	2000HZ	[ ] (LA2000)
	4000HZ	[ ] (LA4000)

Bone conduction necessary? Y/N [ ] (HS)

If so, **R**ight ear, **L**eft ear or **B**oth? [ ]

Comments:

Is there hearing impairment? Y/N [ ] (HC)

Appendix 6: Visual screening proforma

NEURO-COGNITIVE ASSESSMENT STUDY

Visual Screening

Sonksen-Silver Acuity System

Child's Name: _____

Study Number: [N][C][A][ ][ ][ ]

EZHHID: [ ][ ][ ][ ][ ][ ][ ]

Assessor: [ ][ ]

Date of Assessment: [ ][ ]/[ ][ ]/[ ][ ][ ][ ]

Acuity at six metres:	
Both eyes open:	6/ [ ] (BEO)
Right eye:	6/ [ ] (RE)
Left eye:	6/ [ ] (LE)

Classification:	
	[ ] (VS)

Comments:	
Is there visual impairment? (Y/N)	[ ] (VC)

Appendix 7: ERP assessment proforma

NEURO-COGNITIVE ASSESSMENT STUDY

Child's Name: _____ **Sex:** [ ]

Study Number: [ ][ ][ ][ ][ ][ ]

EZHHID: [ ][ ][ ][ ][ ][ ][ ]

DOB: [ ][ ]/[ ][ ]/[ ][ ][ ][ ]

Handedness: [ ]

Date of Assessment: [ ][ ]/[ ][ ]/[ ][ ][ ][ ]

Time of Assessment: _____ to _____

Time of last meal: [ ][ ]:[ ][ ]

Temperature: [ ][ ].[ ] ^{0}C

Pulse rate (per min): [ ][ ]

Is patient taking any drug? (Y/N): [ ]

Assessor: [ ][ ]

Weight: _____

Height: _____

Experiment Summary:

Visual Paradigm done? (Y/N) [ ]

Auditory paradigm done? (Y/N) [ ]

Report: